## A STUDY OF ALBUMIN METABOLISM AMONG NORMAL AND MALNOURISHED FILIPINOS

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Protein-calorie malnutrition is perhaps the most widespread nutritional problem today in the developing countries of the world. The term refers to a continuum of conditions due to deficiency in protein or calories or both, ranging from mild—through moderate—to its most severe forms known as kwashiorkor and nutritional marasmus.(15) Kwashiorkor, due mainly to a deficiency in protein, affects most commonly the weanling child causing growth failure, edema, characteristic hair and skin changes, muscle wasting and psychomotor symptoms. Nutritional marasmus, due to severe protein and calorie under-nutrition, is characterized by growth retardation and extreme wasting of muscle and fat. In between these two clinical syndromes are intermediate cases which cannot be clearly defined as either one or the other. At the same time, lesser degrees of protein-calorie malnutrition referred to as "marginal," "mild-moderate," or "latent," exist and are in fact much more common then advanced syndromes.

It is generally agreed that overt primary protein-calorie malnutrition of the kwashiorkor type is not as common in the Philippines as in some countries of the world. Nutrition surveys in the different regions of the Philippines, however, have revealed that marginal protein-calorie malnutrition does exist, as evidence from dietary and biochemical data.(12) This is also borne out by other data: low growth curves after 6 months

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of life, high morbidity and mortality rates especially among preschool children, etc.

Hypoalbuminemia is the most consistently found biochemical disturbance in protein malnutrition in man. The metabolic mechanisms operating to produce this hypoalbuminemia, however, are still not definitely known. Studies on protein distribution and turnover have been done on severely malnourished children using radioisotopically labelled proteins in an effort to clarify this problem. Thus, Gitlin, et al.[9] and Garrow and Waterlow[8] found that the rate of albumin breakdown in children with kwashiorkor was unaltered, so that they concluded that the hypoalbuminemia was primarily due to decreased albumin synthesis resulting from low protein diet. Picow and waterlow,[20] on the other hand, while admitting a reduction in the rate of protein synthesis prior to therapy, concluded that a reduction in the rate of catabolism is the main factor in bringing about a regeneration of plasma albumin in depleted children. Cohen and Hansen,[6] again found a reduced rate of albumin synthesis in kwashiorkor, but in the presence of an adequate diet, the normal rate of synthesis is promptly restored. Most of these studies were conducted on children suffering or recovering from severe protein-calorie malnutrition of the kwashiorkor type. It was therefore thought important to study these changes among children with milder forms, especially of the marasmic type, for a more complete understanding of this condition. Moreover, since this is the form which is more prevalent in the Philippines and possibly in other developing countries, a study such as this would be of considerable local significance.

The present study therefore is an attempt to determine albumin turnover parameters using [131] I-labelled in children suffering from mild-moderate protein-calorie malnutrition and then again during recovery from such condition. But in order to establish baselines for proper understanding of the changes involved, it was thought necessary to do similar turnover studies on normal Filipino adults and children.

## METHODS

*Labelling of albumin.*—[131] I-labelled albumin was prepared from 20-per cent serum albumin (Behringwerke, Marburg-Lahn) and carrier-free [131] I (Radiochemical Center, Amersham)

essentially by the iodine monochloride method of MaFarlane. (18) In order to obtain consistently good yields, 10-per cent TCA was used as a guide in collecting the albumin from the columns, and the pH was adjusted in both the albumin and the iodide-[131] I-solution to pH 9 to 9.5 just before mixing. Amberlite resin IRA–400(Cl) was used in removing excess iodide. Yields by this method ranged from 69 to 91 per cent (ave. 80 per cent), free iodide content, 0.24 to 0.95 per cent of TCA—precipitable activity (ave. 0.66 per cent), and iodide/albumin ratio, 0.78- to 1.02-g atom/mole (ave. 0.9-g atom/mole). Electrophoresis of the albumin solution with normal serum as carrier yielded 80 to 93 per cent of the radioactivity in the Albumin peak; the rest accounted for by albumin tailing. Final radioactive concentration of the solutions used for injection never exceeded 5 microcurie/mg of albumin. Human albumin was added as carrier to give a final concentration of 1 g per cent. The labelled albumin was sterilized by passing through GS Millipore filter and tested for sterility and freedom from pyrogens.

In five of our subjects, three batches of locally labelled albumin were injected together with IAEA prepared [125] I-albumin. In all cases the results were similar. No significant differences were obtained with the two albumins with respects to plasma volume, half-life, urinary excretion in the first day, and fractional catabolic rate.

Since urinary excretion in the first day may be used as an index of the integrity of the iodinated albumin, it is worth noting that this never went higher than 10 per cent of the injected dose in any of our subjects (range, 2 to 10 per cent).

*Subjects.*—In the study on normal adult Filipinos, 10 healthy young adult males were chosen. Their ages ranged from 20 to 34 years. They were judged healthy by a routine medical and laboratory examination and their weights were within ± 10 per cent of the standard. The subjects were advised to continue with their usual diet and activities.

Nine healthy children (four males and five females) ranging in age from 5 to 8 years were similarly chosen from an orphanage near Manila as subjects for the study in normal children. All were within ± 10 per cent of their standard weight and had serum albumin levels not below 3.5 g per cent. All were ambulatory, afebrile, and not suffering from any constitutional

illness. The children were confined in the orphanage hospital throughout the study and partook of the normal hospital diet.

For the study in malnutrition, eight children (four males and four females) ranging from 4 to 8 years old, and confined in a pediatric hospital near Manila for protein-calorie malnutrition, were chosen for the study. All were grossly underweight with serum albumin concentration less than 3.5 g per cent. None had fever or acute constitutional illness during the study, and none showed edema, hair and skin changes characteristic of kwashiorkor. None could be classified as suffering from either kwashiorkor or, with one exception, severe nutritional marasmus. Body weights and plasma albumin concentrations were more or less constant throughout the weeklong study. All the subjects were admitted to the hospital with an average of 3 weeks earlier either for diarrhea or acute respiratory infection, but at the start of the study, the subject were all pronounced cured from the conditions for which they were admitted. During the study, their diet consisted of approximately 110 cal/kg and 4.7-g protein/kg.

Six months after the first study (Phase I) while the children were recovering from malnutrition, the turnover study was repeated on five of these same children (Phase II). At this time, the subjects increased their weight by an average of 53 per cent of the original, and albumin concentration rose by an average of 24 per cent. During this interval between studies, the children were confined in the hospital, partaking the normal hospital diet (about 70 cal/kg and 3.2 g/kg).

*Procedure of turnover study.*—Two days before and throughout the whole course of the study, the subjects were given Lugol's solution orally, 20 drops per day for the adults and 15 drops per day for the children. [131] I-labelled albumin was injected intravenously to the subjects at a dose of about $\frac{1}{3}$ microcurie per kilo body weight. The amount of the tracer injected was determined by weighing the syringe before and after injection. Standards were similarly prepared and diluted with normal saline and unlabelled serum to give a final radioactive concentration of 0.01 to 0.05 microcurie/ml and a final protein concentration of about 2 mg/ml.

Blood was withdrawn 10 to 15 minutes after injection and then daily thereafter for 7 to 12 days. In adults, blood collection was continued at intervals of 3 to 4 days up to 3 weeks after injection. Twenty-four hours urine samples were also

collected for 7 to 12 days, the end of the collection period coinciding with the collection of the corresponding blood samples.

Blood was withdrawn into oxalated vials and centrifuged immediately. Measured 1- to 3-ml aliquots of standard, plasma and urine samples were counted in a well-type scintillation counter. Samples were counted to at least 10,000 counts or for 20 minutes. Plasma activity was expressed as per cent of injected dose, assuming the 15-minute sample as 98 per cent of dose.(1) Urinary activity was also expressed as per cent of injected dose as derived from counts of standards.

Plasma protein determinations were done in duplicates by the micro-Kjeldahl technique. Values were corrected for NPN. Plasma protein fractions were determined in duplicates by paper electrophoresis using bromphenol blue dye in alcoholic solution,[1] and the strips were analyzed with a Model RB Analytrol. These were made on all blood samples except when hemolyzed, and the average values were taken for computing turnover data. Body weight, body temperature, blood hemoglobin, creatinine and urea nitrogen were measured twice a week.

*Computations.*—Plasma volume (PV) was computed from the usual isotope dilution formula, assuming the activity of the 15-minute plasma sample as 98 per cent of initial activity.

Intravascular albumin mass (IVM) $=$ PV $\times$ plasma albumin concentration.

Extravascular albumin mass (EVM)$=$ ratio of extravascular radioactivity and intravascular radioactivity at equilibrium time (EV/IV), multiplied by IVM.(4)

Total body albumin mass (TBM)$=$IVM $+$ EVM.

Fractional catabolic rate (FCR) was taken as the average of day to day ratios of urinary activity to average activity in plasma on the same day,(5) expressed as per cent of intravascular albumin mass. Urinary excretion data on the first two days was disregarded in computing for FCR. From the absolute catabolic rate, the FCR was also expressed as per cent of TBM.

Absolute catabolic rate (g/d)$=$FRC (in per cent of IVM/d) $\times$ IVM.

Half-clearance time (T$\frac{1}{2}$) (d)—half-life of linear part of plasma curve or of total activity curve extrapolated to zero time.

### RESULTS AND DISCUSSION

*Normal adults.*—A summary of the data obtained from normal adult males is shown in Table 1. All subjects showed a normal plasma albumin concentration ranging from 4.59 to 5.75 g per cent, with an average of 5.04 g per cent. While

TABLE 1.—*Albumin turnover data in 10 normal adult males.*

|  | Range | Mean ±SD |
|---|---|---|
| Age | 20.0 — 34.0 | 27.0 |
| Weight (kg) | 44.7 — 61.9 | 53.8 |
| Plasma volume (liters) | 2.085— 2.757 | 2.438 ± 0.230 |
| (ml/kg) | 40.3 — 49.8 | 45.5 ± 2.9 |
| Albumin concentration (g per cent) | 4.59 — 5.75 | 5.04 ± 0.35 |
| intravascular mass (g) | 102.08 —146.95 | 122.80 ±14.63 |
| (g/kg) | 2.086— 2.804 | 2.287 ± 0.260 |
| Extravascular mass (g) | 115.12 —227.92 | 192.18 ±25.63 |
| (g/kg) | 2.804— 4.350 | 3.595 ± 0.532 |
| Total body mass (g) | 273.90 —374.87 | 314.98 ±35.33 |
| (g/kg) | 4.988— 7.154 | 5.882 ± 0.666 |
| EV/IV | 1.252— 1.810 | 1.571 ± 0.194 |
| Fractional catabolic rate |  |  |
| (per cent IVM/d) | 6.63 — 9.38 | 7.90 ± 0.80 |
| (per cent TBM/d) | 2.32 — 3.57 | 3.08 ± 0.39 |
| Absolute catabolic rate (g/d) | 6.67 — 11.3 | 9.71 ± 1.47 |
| (g/kg/d) | 0.149— 0.218 | 0.181 ± 0.025 |
| Half-clearance time (d) | 18.6 — 31.0 | 22.1 ± 3.4 |

this is higher than some average figures reported in foreign literature,[13, 17, 26] it corresponds closely with an average of 4.98 g per cent reported by Samson, et al.[23] for 87 Filipino males between 20 to 29 years old. The latter authors also showed an average albumin concentration of 68 per cent of the total protein in that same age group, a figure which is close to our (69 per cent). This is slightly higher than the figure of around 60 per cent reported among Americans and Europeans.[17, 21] Whether the differences are real or due to differences in laboratory methods used (the alcoholic method of paper electrophoresis·used in the present work gives higher values for albumin than the aqueous method or salt precipitation [1] remain to be elucidated.

The plasma volume averaged 45.5 ml/kg. Although this may be considered within normal limits, it is slightly higher than the mean value reported by Wasserman, et al.[27] of 42.1 ml/kg using the dye-staff dilution method, or of the results of Steinfield[25] of 42.8 ml/kg among males using [131] I-Albumin.

The average EV/IV ratio among our subjects (1.571) is also higher than the figures reported by Cohen and Schamroth[7]

[1] Spinco Procedure B, Beckman Bulletin 6095-A, November, 1961.

for Africans (ave. 1.4) and European (ave. 1.21). Jarnum(13) reported a distribution ratio of 42 per cent which corresponds to an EV/IV ratio of about 1.38. Cohen, et al.(5) using the equilibrium time method, obtained an average of 1.2.

Because of the differences in albumin concentrations, plasma volumes and EV/IV ratios in our subjects as compared to foreign figures, differences in the albumin masses in the various compartments are to be expected. Thus the intravascular, extravascular and total albumin masses, when expressed in terms of body weight, were higher than the corresponding values reported by Steinfeld,(25) Cohen and Schamroth,(7) Beeken,(3) and Jeejeebhoy.(14) Beeken, et al., for example, found a range of IVM of 1.28 to 2.1 g/kg while Steinfeld reported a mean total circulating albumin of 1.7 g/kg and total exchangeable albumin of 4.55 g/kg. Our mean values for these two parameters were 2.29 and 5.38 g/kg, respectively.

The average fractional catabolic rate in our adult subjects was 7.9 per cent IVM/day, or 3.08 per cent TBM/day. This amounted to 181 mg/kg/day. Jarnum(13) and Beeken, et al.(3) reported slightly higher figures: 10 per cent of IVM/day or 4 to 5 per cent TBM/day, amounting to a catabolic rate of about 200 mg/kg/day. Cohen, et al.(5) obtained an average of 8.8 per cent of intravascular albumin, as derived from urinary/plasma radioactivity data. McFarlane (18) on the other hand summarized normal FCR values in man between 8 to 12 per cent per day of plasma albumin, or a mean absolute catabolic rate of 180 mg/kg/day. It seems therefore that our average value of FCR in terms of intravascular mass is at most in the lower range of normal reported among whites. This observation is also reflected in the longer half-clearance time in our subjects (ave. 22d) than that reported by Jarnum(13) (13 to 17d). Whether these differences are significant remain to be determined. If these differences are true, the lower FCR in our Filipino subjects might reflect an adaptive mechanism to compensate for the generally lower protein intake of the Filipinos. It is interesting to note that among healthy adult Africans, Cohen and Schamroth (7) found an FCR of 8.7 per cent, with an average albumin half-life of 21 days.

*Normal children.*—A summary of the data obtained from nine normal children are shown in Table 2. All the subjects had albumin concentration above 3.5 g per cent with a mean

of 4.08 g per cent. This agreed favorably with the average values reported by Barrion [2] (4 g per cent for age group 5 to 7 years) and by Samson, et al. [23] (3.97 g per cent for age group 1 to 6 years) for Filipino children.

TABLE 2.—*Albumin turnover data in nine normal children.*

| | Range | Mean ±SD |
|---|---|---|
| Age (yrs) | 5.0 — 8.0 | 17.9 |
| Weight (kg) | 15.5 —20.7 | 836.0 ±112.0 |
| Plasma volume (ml) | 661.0 —1017 | 46.5 ± 3.5 |
| (ml/kg) | 42.5 —51.4 | 4.08 ± 0.29 |
| Albumin concentration (g per cent) | 3.57 — 4.56 | 34.16 ± 5.34 |
| Intravascular mass (g) | 24.59 —42.71 | 1.897 ± 0.176 |
| (g/kg) | 1.586— 2.157 | 45.97 ± 6.86 |
| Extravascular mass (g) | 34.42 —55.70 | 2.518 ± 0.354 |
| (g/kg) | 1.989— 3.011 | 79.23 ±10.86 |
| Total body mass (g) | 64.88 —93.26 | 4.415 ± 0.426 |
| (g/kg) | 3.726— 5.041 | 1.302 ± 0.134 |
| EV/IV | 1.130— 1.504 | |
| Fractional catabolic rate | | |
| (per cent IVM/d) | 8.18 —12.62 | 10.90 ± 1.43 |
| (per cent TBM/d) | 3.29 — 6.92 | 4.74 ± 0.90 |
| Absolute catabolic rate (g/d) | 2.58 — 4.81 | 3.70 ± 0.62 |
| (g/kg/d) | 0.166— 0.239 | 0.206 ± 0.026 |
| Half-clearance time(d) | 12.3 —22.0 | 16.4 ± 2.8 |

Plasma volume averaged 46.5 ml/kg, a figure is quite close to the value obtained in our adult subjects. Apparently plasma volumes expressed in terms of body weight does not change much with age. The figure we obtained, however, is slightly lower than those reported by Shah [24] (47.5 to 48.1 ml/kg) in Indian children.

The EV/IV ratio that we obtained averaging 1.302 corresponds to a distribution ratio (IVM/TBM) of about 43 per cent, a figure close to that given by Jarnum [13] (42 per cent). According to Gitlin, et al. [9] the distribution ratio in normal children is about 35 to 50 per cent. Consequently the albumin pool masses in our subjects were apparently all within normal limits.

The fractional catabolic rate in our subjects (ave. 10.9 per cent IVM/day) is higher than the corresponding value that we obtained from our adult subjects by as much as 38 per cent of the adult value. Accordingly the half-clearance time in our young subjects was reduced. The differences in these two parameters between adult and children subjects were highly significant ($p < 0.001$). The absolute catabolic rate among children amounted to 206 mg/kg/day in comparison to the adult figure of 181 mg/kg/day. Apparently, there is a decrease in catabolic rate with advancing age (or increase in body

weight) similar to basal metabolic rate,[18] but whether the figure of weight 0.66 given by Munro [19] relating body weight to albumin turnover rate would apply generally in man remains to be established. If such a figure could be arrived at, it could be a great help in elucidating protein requirements.

*Children suffering from protein-calorie malnutrition.*—The results of turnover studies in eight children suffering from protein-calorie malnutrition are summarized in Table 3. The children ranged from 24 to 53 per cent underweight and had an average plasma albumin concentration of 2.59 g per cent which was only 39.4 per cent of the total plasma proteins. The intra-

TABLE 3.—*Albumin turnover data in eight children suffering from protein-calorie malnutrition.*

| | Range | Mean ±SD |
|---|---|---|
| Age (yrs) | 4.0 — 8.0 | |
| Weight (kg) | 8.5 — 15.6 | 10.2 |
| Per cent underweight | 24.3 — 53.3 | |
| Plasma volume (ml) | 574.0 —897.0 | 669.0 ±97.0 |
| (ml/kg) | 67.5 — 75.1 | 67.5 ± 6.2 |
| Albumin concentration (g per cent) | 1.43 — 3.46 | 2.59 ± 0.74 |
| Intravascular mass (g) | 9.44 — 31.03 | 17.56 ± 6.80 |
| (g/kg) | 0.866— 2.160 | 1.736± 0.533 |
| Extravascular mass (g) | 16.80 — 44.22 | 26.74 ± 8.59 |
| (g/kg) | 1.239— 3.679 | 2.606± 0.700 |
| Total body mass (g) | 26.24 — 75.15 | 44.31 ±14.88 |
| (g/kg) | 2.105— 5.839 | 4.341± 1.152 |
| EV/IV | 1.011— 2.021 | 1.585± 0.299 |
| Fractional catabolic rate | | |
| (Per cent IVM/d) | 6.45 — 10.96 | 8.79 ± 1.70 |
| (Per cent TBM/d) | 2.71 — 4.05 | 3.40 ± 0.52 |
| Absolute catabolic rate (g/d) | 0.711— 2.861 | 1.54 ± 0.486 |
| (g/kg/d) | 0.065— 0.237 | 0.152 ± 0.052 |
| Half-clearance time (d) | 16.7 — 26.0 | 20.0 ± 3.5 |

vascular and extravascular albumin masses were very low in comparison to the values in normal children. It is interesting to note that the average EV/IV ratio was higher in the malnourished children than in the normal children ($p < 0.05$), indicating a shift to the extravascular compartment during malnutrition. This will again be discussed below.

The average plasma volume in ml/kg in our malnourished subjects (67.5 ml/kg) was much higher than in our normal children (46.5 ml/kg). The difference was found statistically significant ($p < 0.001$). This is an agreement with the findings of Waterlow, et al.[28] Bicow and Waterlow,[20] Purves and Hansen,[22] Keys,[16] and Gomez,[10] all pointing to an increase in intravascular hydration during the malnourished state.

The fractional catabolic rate in our malnourished subjects averaged 8.79 per cent of intravascular mass per day, or 3.4 per cent of total body mass per day. The absolute catabolic rate amounted to only 1.54 g/day on the average. These figures are low when compared to the values obtained in the normal children in our series. Thus the FCR (per cent IVM/day) was lower by 19 per cent and the absolute catabolic rate was lower by 58 per cent of the values in normal children. The differences were statistically significant (p < 0.05). This is in agreement with the findings of Picow and Waterlow (20) who reported a mean FCR (per cent IVM/day) of 8.9 per cent in six children suffering from protein-calorie malnutrition. Even lower values were reported by Cohen and Hansen (6) (average 7.1. per cent IVM/day) and Purves and Hansen (22) (range 4.16 to 7.97), but it should be remembered that their subjects were suffering from frank kwashiorkor, while ours mainly belonged to the group designated as mild-moderate protein-calorie malnutrition. It is interesting to speculate that the FCR value is an even more sensitive index of the degree of protein malnutrition than serum albumin concentration. This point will again be touched later.

The mean half-clearance time in our malnourished subjects was also increased in comparison to the normal subjects, and the difference was statistically significant (p < 0.05). This is of course in keeping with the diminished fractional catabolic rate. Picow and Waterlow (20) interpreted this reduction in FCR as a compensatory reaction to the malnutritional state. In malnutrition, the body would conserve protein by reducing its catabolic rate.

*Children recovering from protein-calorie malnutrition.*—The results of the second turnover study on five of our malnourished subjects at the time when they were recovering from malnutrition are compared with the results in these same children during the first study period in Table 4.

It will be noted that the plasma volume expressed in ml/kg diminished from the first to the second study period. Although the average value in the second study period of 54.4 ml/kg did not quite reach the low value in our normal subjects of 46.5 ml/kg, the difference between the values in the first and second study periods was statistically significant (p < 0.05). This again indicates an increased intravascular hydration in the malnourished state.

TABLE 4.—*Albumin turnover data in five children before (Period I) and during (Period II) recovery from protein-caloric malnutrition.*

| | Period of study | |
|---|---|---|
| | I | II |
| Plasma volume (ml) | 680.0 | 839.0 |
| (ml/kg) | 67.2 | 54.4 |
| Albumin concentration (g per cent) | 2.85 | 3.48 |
| Intravascular mass (g) | 19.75 | 29.23 |
| (g/kg) | 1.881 | 1.890 |
| Extravascular mass (g) | 30.67 | 30.24 |
| (g/kg) | 2.918 | 1.957 |
| Total body mass (g) | 50.43 | 59.59 |
| (g/kg) | 4.829 | 3.847 |
| EV/IV | 1.574 | 1.024 |
| Fractional catabolic rate (per cent IVM/d) | 9.10 | 9.469 |
| (Per cent TBM/d) | 3.54 | 4.66 |
| Absolute catabolic rate (g/d) | 1.813 | 2.781 |
| (g/kg/d) | 0.173 | 0.177 |
| Half-clearance time (d) | 19.3 | 18.4 |

While the plasma albumin concentration rose from an average of 2.85 g per cent in the first period to an average of 3.48 g per cent in the second study period, it rose only slightly in three of our subjects (by 0.14, 0.27, and 0.18 g per cent). And while the intravascular albumin mass rose by as much as 48 per cent from the first to the second study period, the extravascular albumin mass remained practically constant. In fact the ratio between extravascular and intravascular compartments diminished significantly from the first to the second study periods. This again points to an apparent shift from the intravascular to the extravascular compartments during malnutrition. This is in contrast to the findings of Cohen and Hansen[6] in their study on kwashiorkor children where they found a proportionately greater reduction in the extravascular than in the intravascular pool in kwashiorkor. Hoffenberg[11] studying the effects of protein depletion in human adults, noted a slight diminution in extravascular/intravascular albumin pool ratio after protein depletion, although the change was not statistically significant. Purves and Hansen;[22] however, did not find this change constantly in their subjects. While it is possible that in mild-moderate depletion states, the EV/IV ratio would indeed be elevated, perhaps minor changes in pool masses, which could not be detected from serial weight or plasma albumin determinations, may account for these discrepancies. Such changes would make the determination of EV/IV ratios by the equilibrium time method difficult. There is a need for further investigation along this line.

The fractional catabolic rate and absolute catabolic rate increased slightly from the first to the second study period, although the differences were not statistically significant. The slight differences may be due to the fact that three of the children were still underweight at the time of the second study (PP—13 per cent, ED—13 per cent, and MC—26 per cent below standard) and two of them had plasma albumin levels below 3.5 g per cent (ED —3.03 g per cent, CB —3.17 g per cent). Furthermore, in at least one of the subjects (MC), the plasma volume was still markedly elevated (70.5 ml/kg), a trend similar to that in malnourished children. In other words, metabolically speaking, the children might not have sufficiently recovered from their nutritional state to show large differences in results even if marked clinical improvement had taken place. It is also possible that the children in the first study period were already in the process of recovering from malnutrition (the change having gone unnoticed probably because it was very slight or because of the short duration of each study period), so that the differences between the two phases of study were not very marked. All these again lend support to the possibility stated earlier: that the FCR value is a more sensitive index of the degree of protein malnutrition than either clinical signs or serum albumin concentration.

Nevertheless, the same trend of increase in FCR and catabolic rate and lowering in half-clearance time was observed among the subjects recovering from malnutrition as in normal subjects. This again supports the contention that in protein malnutrition state, there is a compensatory mechanism to conserve protein by lowering the protein catabolic rate.

## SUMMARY AND CONCLUSION

Albumin turnover studies were done on 10 normal adult Filipinos, nine normal children, and eight children suffering from protein-calorie malnutrition. In addition turnover studies were repeated on five children during recovery from their malnourished state.

Plasma volume in our normal subjects (in ml/kg) were found slightly higher than values reported in foreign literature. On the other hand, the average fractional catabolic rate in our adult subjects was in the lower limit of normal reported among whites. This might reflect an adaptive mechanism to compensate for the generally lower protein intake of the Filipinos.

Normal children, however, were found to have a higher FCR than adults, a trend similar to basal metabolic rate.

Malnourished children showed a markedly elevated plasma volume (in relation to body weight) pointing to an increase in intravascular hydration during the malnourished state. The apparent shift of albumin from the intravascular to the extra-vascular compartments during malnutrition need to be further investigated. The most important finding, however, was a de-crease in fractional catabolic rate during malnutrition, lending support to the contention that in protein depletion states, there is a compensatory mechanism to conserve protein by lowering protein catabolic rate.

During recovery from malnutrition, a return towards normal values was observed. Plasma volume (per kilo body weight) diminished, intravascular albumin mass increased, FCR and absolute catabolic rate increased and half-clearance time diminished. All changes however, were slight, probably because the children might not have sufficiently recovered to show marked difference in results inspite of almost normal albumin concentration and markedly improved clinical appearance, or because the children were already in the process of recovery in the first study period. Perhaps the FCR might therefore be a more sensitive index of the degree of protein malnutrition than either clinical signs or serum albumin concentration.

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# EFFECT OF THE MATURITY OF COCONUT ON THE COMPOSITION AND TEXTURE OF COCONUT FLOUR

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## INTRODUCTION

Coconut flour is produced from coco press-cake which is the by-product of the oil industry. It has a high-protein content of 20 to 25 per cent.[10] From the standpoint of nutrition, this flour can be a good source of protein for food formulations.

While undertaking the development of vitaminised high-protein breakfast food [6] and the study on the incorporation of coconut flour in the preparation of boiled rice, the investigators observed that there was one particular setback in the use of high-level coconut flour as an ingredient—the gritty texture and fibrous feel which is imparted to the product. These sensations experienced by the panel tasters of the Food Research Laboratory (FRL), FNRC, NIST were traced by the investigators to be solely due to the coconut flour.

After developing several formulations containing different levels of coconut flour and subjecting them to FRL panel tasters, it was found that for a product to be acceptable, coconut flour should not be used in excess of 10 to 12 per cent in the formulation.

No published record of any study on coconut meat fiber has been found. Studies were all limited to the proximate analysis of coconut.[1,5,7] Extensive work has also been done on feeding value of copra cake [4] but there is no literature available on its fiber value. Rammamurti and Johar [9] made an attempt to predigest the fibers in coconut press-cake through the use of cellulose of microbial origin. Their communication just gave the method of treatment but the effect on the texture, flavor and nutritive value were not mentioned. Subrahmanyan, however, mentioned that the use of enzymatic digestion in coconut press-cake changes the flavor and also makes the protein content nil after digestion.[8]

The foregoing conditions simply stress the need for conducting a simple and practicable device or procedure that will diminish the gritty texture of the coconut flour and thereby make it possible to incorporate coconut flour at a higher level in food formulations.

The texture of the flour is evaluated by the texture profile method(3) which is defined as the organoleptic analysis of the texture complex of a food in terms of its mechanical and geometrical characteristics and the order in which they appear from the first bite through complete mastication. The texture profile method is a means of obtaining descriptive textural characteristics of food products.

The order in which the texture characteristics are perceived is divided into three phases, namely: (*a*) initial phase as perceived on the first bite; (*b*) masticatory as perceived during chewing; and (*c*) residual as changes during mastication. Textural characteristics are manifested by the reaction of the food to stress while geometrical refers to size and shape of the particles. Phase (*c*) is concerned with the feeling of the product in the mouth during mastication.

## MATERIALS AND METHOD

The coconut flour used in this study was prepared by the investigators from pared coconut meat. This was done because flour from coconuts of different maturity [1] is not available.

Coconuts (Laguna variety [2]) were supplied by the Department of Agronomy, College of Agriculture, University of the Philippines, Laguna. Five stages of maturity (8th to the 12th month old) were harvested from each of 20 coconut trees in one day. The nuts were husked and transported to the Food Research Laboratory, FNRC, NIST.

The coconuts were weighed, cut into halves and the water was collected and weighed. Drying of the coconuts was done by one of the two methods described below. This was made to facilitate the drying of the coconut meat of all stages of maturity and prevent microbial spoilage of the meat. Coconuts of the 8th and 9th month stages of maturity were washed and dipped in 200-ppm sodium metabisulfite solution for 15 minutes,

[1,2] The coconut varieties were identified and maturity established by Mr. J. T. Carlos, assistant head, Department of Agronomy, College of Agriculture, University of the Philippines.

air dried and further dried in a fluidized-bed dryer to a moisture content of about 2 per cent. Those of the 10th, 11th, and 12th months age groups were passed through a Hobart grating machine and then dried immediately in the fluidized-bed dryer. Only one age-group could be dried each day. The other groups were kept in a freezer until ready for drying to prevent any spoilage. Separate weighing of the coconut shell, meat, and water was conducted for each stage (Table 1) before the meat was dried.

Initial extraction of oil from the dried meat in the Carver Press was followed by further extraction of residual oil by the percolation method.[3] Percolation was continued until the solvent extracted meat resisted caking when pulverized in a Willey Mill. The pulverized meat which passed through a 60-mesh sieve was again percolated with hexane until its oil content dropped to about 2 per cent. The residual hexane was subsequently removed upon drying the coco flour, in an infrared oven provided with a blower. This hexane-free flour was ground to 115 mesh in a ball mill.

Proximate analysis was made on samples from each age group. Crude fiber was analyzed in the 60- and 115-mesh flours from each age group. Analysis was conducted according to AOAC procedures.(2)

The texture profile of the five samples of coco flour from the 8th to the 12th month age groups were evaluated by a panel of four technical people chosen on the basis of interest and availability. They also have previous experience in sensory evaluation of food products, which, although not required, is helpful. The panel tasters spent one preliminary session in establishing appropriate reference samples as shown in Table 5a. These selected food items used as reference possess the desired intensity of the textural characteristic and this characteristic is an outstanding property of the selected food and is easy to perceive organoleptically. Once acquainted with the reference samples, each panelist evaluated the test samples independently. After the panelist had evaluated all the sam-

[3] Percolation method—granulated coconut meat contained in a percolator was soaked in hexane. Hexane-oil solution was removed by means of vacuum pump.

ples, one of them acted as a moderator and recorded the end findings.  Any disagreement or misunderstanding was resolved before the session ended so as to arrive at one common measurement of a given textural characteristic.

## RESULTS AND DISCUSSION

Table 1 shows the relative weights of the shell, meat, and water from the 8th to the 12th months age groups of coconut. There was not much change in the weight of the shell.  This may be due to partial desiccation it had undergone while transporting the dehusked nuts from Los Baños, Laguna to

TABLE 1.—*Variation in relative weights of shell, meat, and water at different stages of maturity of coconut.*

| Number of nuts | Age in months | Weight of nuts without husk | Weight of shell | | Weight of water | | Weight of meat | |
|---|---|---|---|---|---|---|---|---|
| | | gm | gm | Per cent | gm | Per cent | gm | Per cent |
| 24 | 8 | 25,257 | 5,178 | 20.5 | 13,815 | 54.7 | 6,264 | 24.8 |
| 36 | 9 | 35,244 | 7,577 | 21.5 | 18,433 | 52.3 | 9,231 | 26.2 |
| 32 | 10 | 29,195 | 6,465 | 21.8 | 11,123 | 38.1 | 11,707 | 40.1 |
| 36 | 11 | 31,367 | 6,744 | 21.5 | 11,449 | 36.5 | 13,174 | 45.0 |
| 28 | 12 | 24,043 | 5,578 | 23.3 | 7,574 | 31.5 | 10,891 | 42.1 |

Manila.  The actual weight of the meat increases as the nut matures.  The increase in the weight of the meat from the 9th to 10th months age group was abrupt and followed by a slight increase in the succeeding stages.  The weight of the water existing as liquid in the nut decreases as it matures. Vesta[11] reported that there is a lowering of the specific gravity of the coconut water as it matures.  The partial desiccation of the shell and the decrease in the weight of the water were not counterbalanced by the increase in the weight of the meat.  This condition must have caused the decrease in the weight of the nut as it matures.

Changes in meat texture and its composition are shown in Table 2.  As the nut ripens the meat losses water and the percentage of dry matter was increased until the nut becomes mature.  The texture of the meat was soft and watery on the 8th month and became crunchy in the last stages of maturity. The 10th month was moist-crunchy while the last two months were dry-crunchy.

TABLE 2.—*Changes in the texture of the meat and composition at different stages of maturity of coconut.*[1]

| Age in months | Texture of meat | Moisture | Ash | Protein | Oil | Total carbohydrate (by difference) |
|---|---|---|---|---|---|---|
| | | Per cent | Per cent | Per cent | Per cent | Per cent |
| 8 | Soft and watery | 89.0 | 0.6 | 2.4 | 3.8 | 4.2 |
| 9 | Soft and leathery | 86.4 | 0.8 | 2.7 | 4.9 | 5.2 |
| 10 | Moist and crunchy | 58.5 | 2.0 | 6.3 | 24.7 | 8.5 |
| 11 | Dry and crunchy | 46.4 | 2.2 | 6.2 | 33.6 | 11.6 |
| 12 | Dry and crunchy | 43.3 | 2.2 | 5.5 | 35.8 | 13.2 |

[1] Analysis was conducted on pared coconut meat.

At the 8th month age, the formation of the oil was small but continued to accumulate until the nut matures. Parallel with the increase in oil was the increase in protein content with slight fluctuations in the more mature groups.

Table 3 shows the amount of flour and its composition for every age group. The amount of flour produced increases from 14 to 25.8 per cent from the 8th to the 12th months age group respectively. The increase from the 11th to the 12th month age group was negligible. Protein in flour (Table 4) does not vary much from the 8th to the 10th months but there was a decrease in the percentage of protein in the more mature

TABLE 3. *Amount of flour produced from each stage of maturity.*

| Number of nuts | Age in months | Weight of flour | Per cent of flour produced[1] |
|---|---|---|---|
| | | gm | |
| 24 | 8 | 880 | 14.0 |
| 36 | 9 | 1,420 | 15.4 |
| 32 | 10 | 2,556 | 21.8 |
| 36 | 11 | 3,344 | 25.4 |
| 28 | 12 | 2,805 | 25.8 |

[1] Per cent of flour produced $= \dfrac{\text{weight of flour}}{\text{weight of fresh meat}} \times 100.$

stage. This finding was the reverse of what was obtained in the coconut meat (Table 2) where the meat from younger coconuts contained less protein than the mature ones. The accumulation of more oil and also the formation of more starch as the nut matures(11) accounts for the decrease in percentage of protein as based on the total nutrient present in the flour. The fiber content of the different age groups with the

same particle size did not vary much. The increase in carbohydrate obtained as the nut matures can be attributed to the increased formation of soluble carbohydrate and not to the fiber content which was observed not to vary so much in the various age groups.

TABLE 4.—*Composition of 60- and 115-mesh coconut flour.*

| Age in months | Moisture | Ash | Oil | Protein | Total carbo-hydrate | Crude fiber | |
|---|---|---|---|---|---|---|---|
| | | | | | | 60 mesh | 115 mesh |
| | Per cent | Per cent | Per cent | Per cent | Per cent | | |
| 8 | 6.5 | 6.0 | 1.4 | 22.9 | 63.2 | 10.3 | 5.4 |
| 9 | 7.0 | 6.3 | 1.4 | 20.0 | 65.3 | 10.5 | 4.3 |
| 10 | 7.2 | 4.7 | 1.5 | 23.3 | 64.3 | 10.6 | 4.3 |
| 11 | 6.9 | 4.0 | 1.4 | 19.9 | 67.8 | 11.0 | 4.6 |
| 12 | 6.8 | 4.0 | 1.4 | 17.7 | 70.1 | 10.7 | 1.5 |

Fresh meat from the more mature coconut gave a more fibrous feeling in the mouth than the meat of the younger ones. This may be attributed in part to the presence of more water in the meat of the younger nut which makes the fibers soft and smooth. The fibrous feeling of the mature nuts can be due to the less water they contain thus making their fibers more rigid and hard. However, there was a marked difference in the fiber content of flour of 60- and 115-mesh sieve particle size. The fiber content of the 115-mesh sieve flour was only half the fiber content of the 60-mesh sieve flour. Since the difference in the fiber content among the different age groups does not vary much when the particle size of the flour is the same, a correlation between texture of the flour and the age of the coconut from which the flour was produced was studied. This was done by conducting a texture profile analysis among different flour samples from each age group.

Table 5 shows the texture profile analysis of the five samples of coconut flour from the 8th to the 12th months age groups. The profile shows the effect of maturity on the texture of the coconut flour produced by each age group. In the initial phase, no difference was noted, all were powdery and adhesiveness was very low. The 8th and 9th months age groups were gummy on chewing, the 10th months age group was slightly gummy and the more mature (11th and 12th months), was not at all gummy. A difference in geometrical characteristic was also noted. The flour from the 8th and 9th months old

TABLE 5.—*Texture profile analysis of five coconut flour samples (115 mesh).*

| | | Age groups, in months | | | | |
|---|---|---|---|---|---|---|
| | | 8th | 9th | 10th | 11th | 12th |
| I. | Initial—perceived on first bite<br>A. Mechanical characteristics—reaction of product to stress which is measured organoleptically by pressure exerted on the teeth, tongue, and roof of the mouth...... | | | | | |
| | Adhesiveness—(1-5 scale)—the work necessary to overcome the attractive forces with which the product comes in contact with tongue, teeth, and palate............. | 2 | 2 | 2 | 2 | 2 |
| | B. Geometrical characteristics—refer to size, shape, and arrangement of particles within the product.......... | powdery | powdery | powdery | powdery | powdery |
| II. | Masticatory — perceived during chewing<br>A. Mechanical characteristics Gumminess (1-5 scale) denseness that persists throughout mastication..... | 3 | 2.5 | 1 | 0 | 0 |
| | B. Geometrical characteristics..... | chalky, lumpy | chalky, lumpy | chalky | chalky, grainy | chalky grainy |
| III. | Residual—changes during mastication<br>A. Rate and type of breakdown.... | Breakdown slowly and unevenly into individual lumps which liquify later | | Breaks slowly and evenly into grainy particles which further breaks into individual grits. | | |
| | B. Moisture absorption............. | Absorbs saliva quickly................ | | Absorbs saliva slowly | | |
| | C. Coating properties.............. | Thick mouth coating.. | | Thin and dry mouth coating. | | |

TABLE 5a.—*Reference standards for texture profile analysis.*

| Standard adhesiveness scale | | Standard gumminess scale | |
|---|---|---|---|
| Panel rating | Reference product | Panel rating | Reference product |
| 1............. | Crisco.................. | 1............. | 40-per cent flour paste |
| 2............. | Dough.................. | 2............. | 45-per cent flour paste |
| 3............. | Cream cheese.......... | 3............. | 50-per cent flour paste |
| 4............. | Marshmallow.......... | 4............. | 55-per cent flour paste |
| 5............. | Peanut butter.......... | 5............. | 60-per cent flour paste |

| Characteristics relating to particle size and shape | Geometrical characteristics of texture | | Reference product |
|---|---|---|---|
| | Reference product | Characteristics relating to shape and orientation | |
| Powdery........ | Johnson baby powder........... | Flaky.......... | Corn flakes |
| Grainy.......... | Cream of wheat............... | Fibrous........ | Chopped boiled breast of chicken or base of asparagus shoot |
| Gritty.......... | Very fine sand............... | ............... | |
| Coarse.......... | Cooked oatmeal............... | ............... | |
| Lumpy.......... | Native cheese cut into ½ cm. square | Cellular...... | Raw apple or sponge cake...... |
| Beady.......... | Boiled sago................... | Aerated........ | Milk shake |
| ............... | ............... | Puffy.......... | Puffed rice |
| ............... | ............... | Crystalline..... | Granulated sugar |

coconuts were chalky which later became lumpy; on the 10th month just chalky; and from the 11th to 12th months chalky which later turned grainy. The breakdown characteristic of the 8th and 9th months old samples was the formation of individual lumps as a result by their quick absorption of saliva and production of a thick coating in the mouth. The flour from the 10th to the 12th month age groups showed slow absorption of saliva and then turned to individual grits. They coated the mouth thinly and gave a dry feeling. There was no fibrous feeling in any of the samples tested.

## SUMMARY AND CONCLUSION

Coconut flour was produced from coconuts varying from the 8th to 12th months stage of maturity. Proximate analysis was conducted on the pared coconut meat samples and the flour produced from them. A classification of textural characteristics was defined for each class of flour by the use of texture profile analysis.

There was an abrupt increase in oil content and flour yield on the 10th month age with slight increase as the nut matures. Texture of the meat for each age group changed from soft-watery for young nuts to dry-crunchy for mature nuts. The 10th month age group was different from the rest in that it was moist-crunchy which probably makes the flour of this age neither lumpy nor grainy. Nutrition wise, the 10th-month age group is the best picking stage since it is, at this age where maximum protein content was attained and after which the protein decreased slowly as the nut matures. There was very little difference in fiber content at the various stages of maturity. However, fiber content is related to particle size of the flour as shown by the difference in fiber values of the 60- and 115-mesh flour. Texture profile analysis showed that flour from the 11th to 12th months age groups of coconut became gritty in the mouth while those from the 8th and 9th months age groups became lumpy later turning watery. There was no fibrous feeling in any of the sample group.

It was observed that the fibrous feeling encountered by the investigators in their use of more than 10- to 12-per cent coconut flour is not due to fiber content but may be attributed to the composition of the flour itself. The grinding of coconut flour to a finer mesh did not remove the gritty feeling.

The fiber content of the flour of smaller particle size was lower than those of bigger particle size.

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# PREPARATION AND STORAGE QUALITIES OF FORTIFIED NATA DE COCO

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TWO TEXT FIGURES

## INTRODUCTION

*Nata de coco* is a mass of gelatinous substance which forms on the surface of coconut water medium produced by some bacteria of which opinions of several workers (1, 11, 15) vary as to its identity.

Valuable data on coconut water are available. Peters(17) isolated and identified the B-group vitamins present in coconut water. The corresponding amounts of these vitamins in each cubic centimeter of coconut water are: nicotinic acid, 0.01 microgram; biotin, 0.02 microgram; panthothenic acid, 0.52 microgram; riboflavin, 0.01 microgram; folic acid, 0.003 microgram. Miller(12) found coconut water to have the following composition: protein, 0.23 per cent; carbohydrates, 3.68 per cent; fat, 3.56 per cent; calcium, 0.03 per cent; and phosphorus, 0.01 per cent. Intengan, et al.(7) reported the composition of coconut water as follows: protein, traces; fat, 0.2 per cent; carbohydrates, 5.1 per cent; ash, 0.3 per cent; calcium, 16 per cent; phosphorus, 6 per cent; iron, 0.2 per cent; and traces of thiamine, riboflavin, niacin, and absorbic acid. Caray(5) identified and isolated sucrose, dextrose and fructose as sugars present in coconut water. It is probable that the presence of these nutrients stimulates the growth of the organism that produces *nata* in coconut water.

Analysis of nata de coco cooked in syrup, also known as nata de coco preserve,(14) shows that it contains water, 67.7 per cent; protein, nil; fat, 0.2 per cent; calcium, 12 mg per cent; iron, 5 mg per cent; phosphorus, 2 mg per cent; thiamine, traces, riboflavin, 0.01 microgram per cent.

The above review shows that only a few of the nutritive constituents of coconut water are transmitted to the nata. Nata de coco preserve may therefore be considered as a food without

nutritive value, although it ranks as a food of major importance when "consumers' appetite" is concerned.

Nata de coco preserve is considered a delicacy among Filipinos. The cost involved in its preparation as well as the price of the commercial processed product are within the reach of the major segment of the population.

Fortification of nata de coco preserve with some vitamins and essential minerals will make this product more beneficial to consumers as its nutritive value will be enhanced and its flavor, texture, and color improved. It will also help bolster the vitamin and mineral intakes of our people which will in a way alleviate the malnutrition problems of the country. It will also appeal to nutrition-conscious consumers who are appreciative of the widening variety of foods which provide them with better nutrition.

This study was conducted (*a*) to study the possibility of increasing the nutritional quality of nata de coco preserve without impairing the degree of preference of the consumer for this food and (*b*) to determine the effect of storage on the nutrient composition in the liquid and solid phases and acceptability of the fortified nata de coco.

## MATERIALS AND METHODS

The method used is a modification of the traditional method of processing nata de coco preserve. The traditional method for processing is briefly described below.

The newly harvested nata de coco is cleaned and sliced to a desired size. It is then boiled with constant changing of water until the product is free from acid flavor. The acid-free product is drained and boiled with syrup. The sugar impregnated nata is bottled, exhausted, sterilized, and packed.

The steps modified in the traditional method are: leaching of the nata, syruping, packaging, and an additional step of fortification.

*Leaching of raw nata.*—Boiling of the raw nata with water is the method used in leaching the acids accumulated in the nata during its formation by the microorganism. According to our findings, the conventional method of continuous boiling causes the browning of the product. This method was modified in this study. In this modified procedure, the clean and sliced product was boiled for five minutes, immediately washed with water and soaked in tap water overnight. This

step was repeated daily until the product was free from acid flavor.

*Syruping.*—It was observed that the concentration of the syrup of the preserved nata decreases rapidly until the syrup becomes watery on storage. It is therefore necessary to equilibrate the syrup of the nata before subjecting to fortification. In this way, any change in sugar concentration after fortification will not be caused by diffusion of sugar to the nata but rather it may just be attributed to the effect of the added nutrients.

The modified method of syruping was to mix the nata de coco with extra heavy syrup (2:1, sugar: water) and boiled for five minutes. The resulting mixture gives a syrup with 76-per cent soluble solids. The boiled product in syrup was allowed to cool at room temperature and kept in a refrigerator to prevent the onset of any fermentation. This step was repeated daily until the syrup concentration of 45° to 50° Brix was attained for two consecutive readings at 12 hours interval. Whenever the sugar concentration fell below the working standard of 45° to 50° Brix before the concentration got stabilized, sugar was added until the concentration was within the working standard.

*Fortification.*—The sugar-impregnated nata de coco was drained of its syrup. The drained nata was weiged. A newly prepared syrup (1:1 sugar: water) was added to the syrup from the drained nata until its weight was equal to the drained nata. The syrup was boiled in a stainless steel container until a clear syrup was obtained. The impurities which coagulated were removed by skimming. The syrup was further clarified by filtration through a cheese cloth and was then fortified with the vitamins, niacin, thiamine, riboflavin, ascorbic acid, and minerals calcium and phosphorus.

The calculated amounts of nutrients based on a well-blended flavor as evaluated by the flavor profile method of Cairncross(4) were added to the syrup. Calcium di-phosphate was added to the boiling syrup, stirred until dissolved and cooled to a temperature of 50° to 60° C. At this temperature of the syrup, all the vitamins, in the order of their stability to the existing conditions, were added as follows: niacin, thiamine, riboflavin, and ascorbic acid. The container was covered with opening just enough for the stainless steel ladle to move around. This will prevent the entrance of light and incorporation of too much air

in the syrup during stirring. Drops of food flavoring were added. The flavoring used in this study was *kalamansi* (*Citrus microcarpa* Bunge) oil.[1]

*Packaging.*—The conventional way of bottling nata de coco preserve is the use of clear glass jars. In fortified nata de coco, these clear glass jars must be further wrapped to prevent absorption of light by the fortified product. In this study, the fortified nata de coco was bottled in a clear glass jar and wrapped in dark green cellophane. Dark colored cellophane was used so that the product is visible, otherwise ordinary Manila paper wrapping might serve the purpose. If amber glass jars or enameled cans are used, wrappings may not be necessary.

*Storage studies.*—Analysis of the fortified nata de coco: solid and liquid portions combined; liquid portion only, and drained nata only were conducted after processing to determine the per cent retention of the added nutrients.

All the bottled fortified nata de coco samples were divided into two lots. One lot was stored in a shelf at room temperature (30° C) and the other in a household refrigerator (4° C). At monthly intervals up to 11 months, random samples of four bottled fortified nata de coco were taken from each storage condition. In order to determine if the nata (solid portion) had absorbed the nutrients, two of the bottled nata de coco from each storage condition were drained thoroughly of its syrup. The solid and liquid portions were separately subjected to objective quality evaluation as pH of the syrup; soluble solids and chemical analysis for the added nutrients as thiamine, riboflavin, niacin, ascorbic acid, calcium, and phosphorus.

The rest of the bottled fortified nata de coco from the two storage conditions were used for acceptability test of the product.

The following methods were used for analysis:

pH: The pH of the syrup was read on a Beckman pH meter model No. G.

Soluble solids: Soluble solid content of the syrup was measured with a Zeiss-Opton hand refractometer. The results were reported as per cent soluble solids.

Chemical analysis: Thiamine was measured by the Hennessey and Cerecedo thiochrome method as reported by Munsell, et al.(13) Riboflavin was measured by the Hodson and Norris fluorometric method.(6) Niacin

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[1] Kalamansi oil was supplied by Mrs. Luz V. Adeva of the Food Research Laboratory.

was measured by the U.S. Pharmacopoeia microbiological method.(16) Ascorbic acid was measured by the Bolin and Book modification of Roe and Oesterling colorimetric method.(3) Calcium was determined by Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.(2) Phosphorus was determined by Lowry and Lopez modification of the Fiske and Subbarow method.(10)

*Acceptability test.*—Samples from each storage condition were evaluated by paired comparison,(9) by a taste panel of 26 persons. These panel tasters are members of the technical staff of the Food Research Laboratory, FNRC, NIST and have the ability to recognize off-flavor characteristics of the product. In conducting the acceptability test, each taster received a tray containing two samples of fortified product—one of which was stored under room temperature storage and the other from refrigerated storage. Each sample was put in an amber cup container. Amber cups were used to mask all the other attributes of the product except flavor and aroma. Flavor and aroma were the sensations evaluated by the panel tasters as the product aged in each storage condition. The amber cups were coded with a three-digit number. Attached to each tray was a paper containing instructions as follows:

Name ............................ Date ............................ Time ............................
Taste each sample and indicate which one you prefer.
You must make a choice, even if only a guess.
The responses were tested statistically using the "t" test.

## RESULTS AND DISCUSSION

*Retention of nutrients.*—Table 1 shows the nutrient composition of the fortified nata preserve before and after processing. Thiamine being heat-labile and riboflavin which is light-labile, were the vitamins most affected by the processing conditions, showing 89- and 97-per cent retention, respectively. The investigators could not attribute the loss of thiamine to the temperature of the syrup because thiamine is considered stable up to one hour at 100° C and at pH 3.5. We may attribute the loss of thiamine to the pH of the syrup which was 6.25 at the time it was added and did not decrease until after processing. Niacin was very stable under conditions encountered in processing so that its loss was negligible, giving 94-per cent retention. Ascorbic acid, being the last nutrient added was therefore least exposed to oxygen in the air. Furthermore, the temperature of the syrup was at its minimum when it was added. Hence a 96-per cent retention was attained.

TABLE 1.—*Per cent retention of nutrients after processing fortified nata de coco.*

| Nutrients | Unfortified nata de coco | Fortified nata de coco | | Retention |
|---|---|---|---|---|
| | | a Amount dded before processing | Amount soon after processing | |
| | *mg 100 g* | *mg 100 g* | *mg 100 g* | *Per cent* |
| Niacin | nil | 7.522 | 7.070 | 94 |
| Thiamine | traces | 0.6443 | 0.5748 | 89 |
| Riboflavin | 0.0100 | 0.3682 | 0.3298 | 87 |
| Ascorbic acid | nil | 27.61 | 26.62 | 96 |
| Calcium | 12 | 62.86 | 67.63 | 89 |
| Phosphorus | 5 | 95.14 | 71.00 | 73 |

Calcium and phosphorus were added in the form of calcium diphosphate, a very stable substance and soluble in water. Since the solvent was a heavy syrup in this process, its solubility was lower. This low retention of calcium (89 per cent) and phosphorus (73 per cent) could have been caused by some undissolved calcium di-phosphate that may have settled at the bottom of the mixer.

The pH of the samples (Fig. 1) stored under the two storage conditions (refrigerated and under room temperature) was always on the acid side up to the end of the experimental study. The acidic reactions observed might be attributed to the fact that the aqueous solution of the nutrients added were acid.

After the first month storage, the pH of samples stored at refrigerated and at room temperature conditions were almost steady at pH 4.5 with sudden lowering after the fourth month storage. This sudden change could be due to weather condition (typhoon season) with interrupted electricity so that most of the time, both storage studies were subjected to the same condition from the third to the fourth months. After the seventh month up to the end of the study the pH remained stable at pH 3.5 in the two storage conditions. The slight increase of the pH to 4.1 on the eleventh month on the sample at room temperature storage might be caused by the varied room temperature (28° to 35° C) between the tenth and eleventh month storage. The maintenance of a low pH during storage was favorable to a high retention of vitamins.(8)

*Distribution of nutrients in the solid and liquid phase.*—The customary way of eating nata preserve is to eat the solid portion with little or no syrup at all. The nutrient content of the product at the time they are eaten is the most important
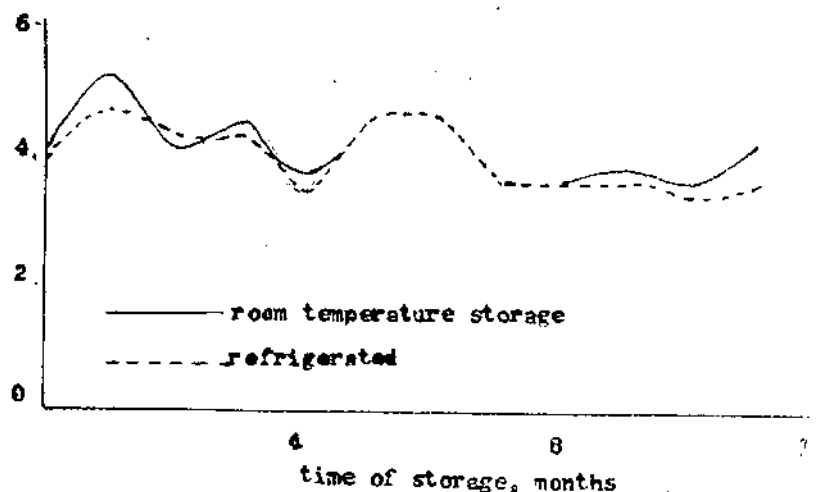
FIG. 1. Changes in pH of the fortified nata de coco stored under two storage conditions (refrigerated and under room temperature).

consideration from the standpoint of consumers' health. Since the fortified nata de coco is syrup-packed and only the syrup was fortified, the solid and liquid phase distribution of the nutrients is a very important consideration to verify. All the nutrients added were water-soluble so that a good picture of such distribution could be obtained by determining the nutrient retention during storage in the liquid and solid phases independently.

Tables 2 and 3 show the distribution of nutrients in the solid and liquid phases during the eleventh-month storage. After processing (0 time), the added nutrients were almost equally distributed between the solid and liquid phases, except for niacin and riboflavin. From the first month storage up to the end of the experimental studies, the nutrient distribution in the two phases was no longer the same as the initial distribution on both storage conditions. There was negligible decrease of nutrient levels in the samples stored at refrigerated condition. This may be due to the controlled temperature (4° C) storage. Also, the low pH would explain the negligible loss of some vitamins since vitamins are more stable under this condition.(8) The riboflavin and ascorbic acid levels in the solid and liquid phase decreased in the samples stored at room temperature. This may be due to the oxidizing property of the riboflavin. The small percentage decreases and increases of

TABLE 2.—*Distribution of nutrients in the liquid and solid phase of fortified nata de coco during storage at refrigerated temperature ($4°C$) for 11 months.*

(Time of storage, months.)

| Nutrients mg/100 gm | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Thiamine:** | | | | | | | | | | | | |
| Solid phase | 0.47 | 0.44 | 0.48 | 0.56 | 0.53 | 0.48 | 0.44 | 0.52 | 0.53 | 0.52 | 0.54 | 0.56 |
| Liquid phase | 0.52 | 0.42 | 0.49 | 0.45 | 0.42 | 0.43 | 0.52 | 0.50 | 0.46 | 0.54 | 0.51 | 0.54 |
| **Riboflavin:** | | | | | | | | | | | | |
| Solid phase | 0.41 | 0.34 | 0.34 | 0.33 | 0.24 | 0.23 | 0.17 | 0.22 | 0.24 | 0.29 | 0.29 | 0.26 |
| Liquid phase | 0.20 | 0.29 | 0.29 | 0.32 | 0.31 | 0.21 | 0.16 | 0.13 | 0.14 | 0.19 | 0.19 | 0.24 |
| **Niacin:** | | | | | | | | | | | | |
| Solid phase | 4.1 | 5.0 | 4.6 | 5.0 | 4.7 | 5.4 | 4.6 | 5.6 | 5.1 | 5.5 | 4.5 | 5.6 |
| Liquid phase | 9.1 | 5.8 | 4.9 | 5.1 | 5.7 | 5.5 | 5.5 | 5.5 | 5.2 | 5.7 | 4.6 | 5.0 |
| **Ascorbic acid:** | | | | | | | | | | | | |
| Solid phase | 26.0 | 17.0 | 26.0 | 22.0 | 22.0 | 25.0 | 21.0 | 20.0 | 22.0 | 20.0 | 20.0 | 24.0 |
| Liquid phase | 30.0 | 25.0 | 22.0 | 21.0 | 28.0 | 22.0 | 24.0 | 18.0 | 21.0 | 23.0 | 24.0 | 25.0 |
| **Calcium:** | | | | | | | | | | | | |
| Solid phase | 47.0 | 63.0 | 49.0 | 84.0 | 77.0 | 32.0 | 34.0 | 42.0 | 47.0 | 44.0 | 59.0 | 61.0 |
| Liquid phase | 43.0 | 31.0 | 95.0 | 61.0 | 71.0 | 38.0 | 26.0 | 37.0 | 33.0 | 36.0 | 49.0 | 50.0 |
| **Phosphorus:** | | | | | | | | | | | | |
| Solid phase | 33.0 | 33.0 | 33.0 | 41.0 | 28.0 | 31.0 | 38.0 | 28.0 | 34.0 | 30.0 | 28.0 | 37.0 |
| Liquid phase | 34.0 | 26.0 | 28.0 | 45.0 | 20.0 | 35.0 | 30.0 | 26.0 | 29.0 | 28.0 | 30.0 | 34.0 |
| pH | 3.8 | 4.6 | 4.2 | 4.2 | 3.4 | 4.5 | 4.5 | 3.5 | 3.5 | 3.5 | 3.3 | 3.5 |
| Soluble solids (°Brix) | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | 48.5 | 48.0 | 48.5 | 49.5 | 48.0 | 48.0 | 47.5 |

TABLE 3.—*Nutrient contents of fortified nata de coco during storage at room temperature (30°C) for 11 months.*

(Time of Storage, months.)

| Nutrients mg/100 gm | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Thiamine:** | | | | | | | | | | | | |
| Solid phase | 0.47 | 0.44 | 0.46 | 0.41 | 0.38 | 0.41 | 0.44 | 0.51 | 0.51 | 0.50 | 0.44 | 0.49 |
| Liquid phase | 0.52 | 0.43 | 0.48 | 0.44 | 0.44 | 0.38 | 0.48 | 0.49 | 0.49 | 0.51 | 0.45 | 0.48 |
| **Riboflavin:** | | | | | | | | | | | | |
| Solid phase | 0.41 | 0.35 | 0.34 | 0.25 | 0.29 | 0.21 | 0.22 | 0.18 | 0.21 | 0.18 | 0.18 | 0.16 |
| Liquid phase | 0.20 | 0.29 | 0.33 | 0.33 | 0.31 | 0.20 | 0.17 | 0.15 | 0.16 | 0.19 | 0.16 | 0.19 |
| **Niacin:** | | | | | | | | | | | | |
| Solid phase | 4.1 | 5.0 | 7.1 | 4.9 | 4.7 | 5.7 | 3.7 | 5.9 | 4.7 | 5.3 | 4.8 | 6.5 |
| Liquid phase | 9.1 | 7.1 | 4.6 | 5.2 | 6.2 | 5.5 | 5.0 | 5.5 | 5.2 | 5.4 | 4.7 | 4.7 |
| **Ascorbic acid:** | | | | | | | | | | | | |
| Solid phase | 26.0 | 20.0 | 27.0 | 22.0 | 20.0 | 13.0 | 16.0 | 14.0 | 15.0 | 13.0 | 14.0 | 14.0 |
| Liquid phase | 30.0 | 22.0 | 27.0 | 20.0 | 23.0 | 20.0 | 18.0 | 14.0 | 17.0 | 18.0 | 16.0 | 15.0 |
| **Calcium:** | | | | | | | | | | | | |
| Solid phase | 47.0 | 63.0 | 45.0 | 37.0 | 68.0 | 28.0 | 40.0 | 45.0 | 45.0 | 46.0 | 58.0 | 53.0 |
| Liquid phase | 43.0 | 32.0 | 94.0 | 63.0 | 60.0 | 41.0 | 30.0 | 33.0 | 38.0 | 37.0 | 47.0 | 39.0 |
| **Phosphorus:** | | | | | | | | | | | | |
| Solid phase | 33.0 | 43.0 | 31.0 | 43.0 | 24.0 | 27.0 | 35.0 | 25.0 | 35.0 | 33.0 | 50.0 | 36.0 |
| Liquid phase | 34.0 | 26.0 | 29.0 | 46.0 | 20.0 | 34.0 | 27.0 | 30.0 | 26.0 | 26.0 | 34.0 | 32.0 |
| pH | 3.8 | 5.1 | 4.0 | 4.4 | 3.6 | 4.5 | 4.5 | 3.5 | 3.5 | 3.7 | 3.5 | 4.1 |
| Soluble solids (°Brix) | 49.0 | 49.0 | 49.0 | 49.0 | 49.0 | 48.5 | 48.0 | 49.0 | 49.0 | 48.0 | 48.6 | 45.0 |

nutrients in both phases under the two storage conditions could not be explained since the total nutrient content (solid and liquid) of the whole *nata* preserve was not determined during its monthly analysis.

The movement of the nutrients in the two phases followed the same trend. That is, there was an increase in concentration in the liquid or solid phase until a time when the concentration in the two phases was the same. After this point, the concentration reversed in the two phases. This shows that the nutrients migrate from the liquid to solid and back in a continuous cycle with different migration velocities for each nutrient.

In terms of our observations, we may describe nata as the solid phase which acts as an adsorbent and the added nutrients as the adsorbable substances (adsorptives). When the solution of nutrients was brought into contact with an adsorbent (solid phase), molecules of the adsorptive pass out of the solution into the interfacial region of the adsorbent and were retained for a longer or shorter time depending on the strength of adsorption on each nutrient. As the concentration of each nutrient builds up in the interface of the solid phase, the solution is depleted of the nutrients. But by reverse process, the escape of nutrients from interface into solution is also occuring and eventually an equilibrium state was reached when the solution and solid phases had equal concentration of nutrients. The state of equilibrium was only for a short time and desorption or adsorption of nutrients starts again.

In the two storage conditions, calcium had a very irregular movement in the two phases up to the fifth month before it became more or less stable. The movement of phosphorus in the two phases was more uniform as compared to calcium. Except for the bahavior of riboflavin in samples stored in the refrigerator, all the nutrients in the two phases might be considered stable after the seventh month.

The soluble solids (Tables 2 and 3) in the syrup which may be considered as its sugar concentration remains constant up to the end of the eleventh month storage. This shows that the sugar concentration was well stabilized before fortification. The movement of nutrients in the two phases may be considered independent of the stable sugar concentration.

*Acceptability test.*—Results of the acceptability tests were analyzed statistically using the "t" test. These are shown in

Table 4. The absolute value of "t" was 1.03 which is less than the t-value of 2.086 at 95-per cent confidence. Hence, it can be concluded that there was no significant difference in the acceptability of the product stored at room temperature or under refrigerated condition.

TABLE 4.—*Acceptability test of the fortified nata de coco under two storage conditions.*

| Time in months | Room temperature storage | Refrigerated temperature storage |
|---|---|---|
| | (*Number of tasters accepting the product*) | (*Number of tasters accepting the product*) |
| 1 | 19 | 7 |
| 2 | 18 | 8 |
| 3 | 16 | 10 |
| 4 | 14 | 12 |
| 5 | 14 | 12 |
| 6 | 20 | 6 |
| 7 | 17 | 9 |
| 8 | 11 | 15 |
| 9 | 11 | 15 |
| 10 | 6 | 20 |
| 11 | 8 | 18 |

Statistical analysis by "t" test

$t = 1.03$          for d.f. $= 20$          $t.05 = 2.086$          ∴ it is not significant

Having obtained no significant results by using the t-test, the investigators tried another method—a graphical analysis of the taste panel responses. The three-month moving average of the number of tasters preferring fortified nata stored under each condition (i.e., stored at room temperature and refrigerated temperature) were plotted against storage time (Fig. 2).

It may be observed from the graph that there is decidedly a stronger preference among the panel of tasters for fortified nata stored at room temperature up to the seventh month. Starting the seventh month, the preference for that stored at room temperature showed a rapid decline, while that stored by refrigerated temperature increased. On the ninth month, there was equal preference for the fortified nata stored under both conditions. Thereafter preference for that stored at room temperature continuously declined while that stored at refrigerated temperature continuously increased. This definite reversal of preference among panel tasters for fortified nata stored at refrigerated temperature may be attributed to the stability of nutrient levels in the liquid and solid phases as was mentioned earlier. The stability of the nutrients levels may have influenced the formation of a complete blend of nutrients resulting in a more desirable flavor and aroma.

time of storage, months
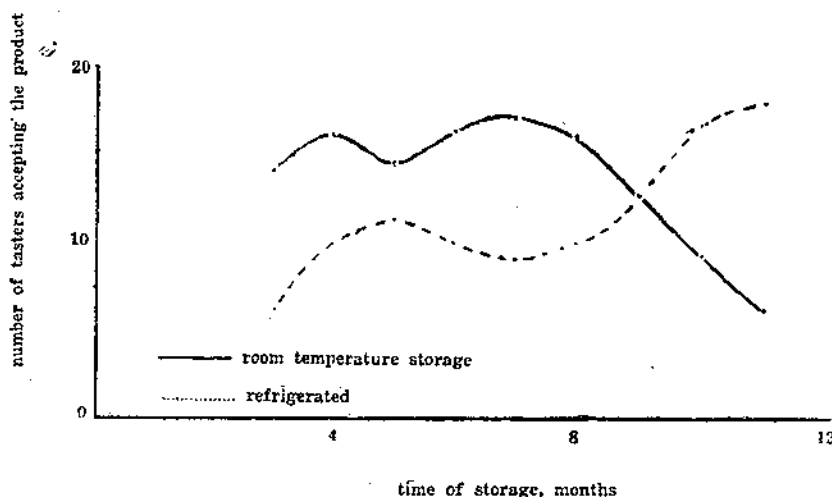
FIG. 2. Graphical analysis of the taste panel responses on fortified nata de coco stored under two storage conditions (refrigerated and under room temperature).

## SUMMARY AND CONCLUSION

A newly harvested nata de coco was cleaned, deacidified and impregnated with sugar. The sugar-impregnated nata was drained of its syrup and the syrup was fortified with the following vitamins and minerals: niacin, 7.522 mg/100 gm; riboflavin, 0.3682 mg/100 gm; thiamine, 0.6443 mg/100 gm; ascorbic acid, 27.61 mg/100 gm; calcium, 62.86 mg/100 gm; and phosphorus 95.14 mg/100 gm. The sugar-impregnated nata with an equal weight of fortified syrup was bottled and processed. Thereafter a shelf-life study was conducted for 11 months on two storage conditions (refrigerated and room temperature storage.)

It was observed that after processing, niacin and ascorbic acid suffered negligible loss giving 94- and 96-per cent retention, respectively. The rest of the nutrients had the following per cent retention: thiamine, 89 per cent; riboflavin, 87 per cent; calcium, 89 per cent; and phosphorus, 73 per cent. The sugar concentration was constant during the experimental study. pH value was on the acid side and was steady at pH 3.5 after the seventh month in both storage conditions with a slight increase at the last month storage on the samples stored at room temperature. During storage the nutrients migrated

from liquid to solid and back in a continuous cycle with different migration velocities. The fortified nata was acceptable to the panel tasters.

It is possible to improve and increase the nutritional quality of nata de coco by fortifying it with vitamins and minerals without impairing the degree of preference of consumers for the food. The nutrients were more stable when samples were stored at constant low temperature (4° C). Nata and syrup both contain nutrients at any time during the 11 months shelf-life study under the two storage conditions. Fortified nata, whether stored at room temperature or refrigerated, were both acceptable to the panel tasters. However, there is a greater degree of preference for fortified nata stored at room temperature over that stored at refrigerated temperature up to the ninth month. Thereafter, fortified nata stored under refrigerated temperature became more preferred.

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# THE EFFECT OF TEMPERATURE AND TIME OF STORAGE ON THE NUTRITIVE VALUE AND ACCEPTABILITY OF FORTIFIED CANNED MANGO NECTAR

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TWO TEXT FIGURES

## INTRODUCTION

The preparation of canned or bottled fruit juices is one of the most significant developments in the food industry. Besides providing nutritious beverages, they also serve as an additional outlet in the utilization and marketing of fruit. Nectars (pulpy fruit juices blended with sugar syrup) from apricots, peaches, pears and prunes have gain wide acceptance in the western countries. Several reports have been published on the preparation and storage of such products.(4, 7, 11, 12)

Small amounts of guava, passion fruit, and banana nectars are packed in Hawaii.(1) Mango nectar was recently introduced in Puerto Rico.(17) In India, the preparation and preservation of nectars from jackfruit and mango have been reported.(5, 13)

Juices differ markedly in their respective rates of deterioration in storage. This deterioration is confined for the greater part to undesirable changes in flavor, appearance and nutritive value. Several fruit nectars or pulpy juices are being commercially packed but little information is available on the vitamin retention in these products during storage.

In recent years, the addition of ascorbic acid to a wide variety of canned products has been done.(2, 8, 16) The added ascorbic acid has served to enhance the nutritive value as well as to prevent or retard browning and off-flavor development.(8, 19)

Studies on the effect of canning and storage on the B-carotene content of mango, papaya, and jackfruit showed that carotene retention after 5½ months storage at room temperature was 61.7 per cent.(18) Other investigators (6, 10, 14) have likewise studied the stability of carotene in commercially canned foods during a storage up to 110° F. They have reported good

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carotene retention in the food samples studied, except in tomato juice, yellow corn, orange juice and green beans where significant losses occurred following 18 months storage at 70° F. It should be borne in mind, however, that individual foods differ in their ability to retain the same nutrient.

Mango is one of the most widely acceptable seasonal fruits in the Philippines. The ripe fruit is highly perishable. There are several varieties grown in the Philippines but the *carabao* variety is the most widely grown and the most delicious among them. It is rich in carotenoid pigments, containing on the average 2580 I.U. of vitamin A per 100 grams of edible portion.[9]

This study was undertaken to determine the preservation and storage qualities of canned fortified mango nectar with respect to acceptability and retention of nutritive value.

### EXPERIMENTAL PROCEDURE

Mango nectar was prepared by the following procedure: Fully ripe, sound fruits of the carabao variety were thoroughly washed with detergent, then carefully rinsed in running water. The fruits were peeled and then cut lengthwise with a stainless steel knife on either side of the seed, thus obtaining two large sections which contain most of the pulp. The central section containing a little more pulp over the seed was scraped slightly. The pulp was then mashed in a Waring blendor to obtain a fine and smooth puree. The puree was then sweetened with a equal quantity of 18° Brix sugar syrup acidified with 0.3-per cent citric acid. The resulting mixture was treated with sodium benzoate to give a final concentration of 0.05 per cent and pasteurized at 85° C for 5 minutes. Ascorbic acid equivalent to 50 mg per 100 ml of nectar was added as soon as the temperature of the pasteurized juice reached 85° C or just prior to the canning process. The hot pasteurized juice was then filled into previously sterilized plain tin cans (No. 1 tall 301 × 411), sealed completely and processed for 10 minutes in boiling water. They were then cooled immediately under running water.

The finished products were divided into 2 lots, one was stored at room temperature (29° to 32°C) and the other in household refrigerator (4° to 5°C) for storage studies.

### EXAMINATION OF JUICES

Ascorbic acid determination of the fortified nectar before and after fortification and during storage for a period of 12

months at periodic intervals was done using the indophenol dye titration method.(3)

Titratable acidity (expressed as per cent anhydrous citric acid) was determined by titration with 0.1N sodium hydroxide using phenolphthalein as indicator on the prepared nectar after processing and also at regular 2-month intervals for one year. The pH value of the freshly processed sample was determined by means of a Beckman pH meter. The total soluble solids, expressed in degrees Brix, was taken using the Zeiss-Opton hand refractometer.

Carotene determinations were made on the freshly processed and stored products using the Wall and Kelley modification of Moore chromatographic method.(21)

Color determinations were made on the freshly processed and stored products by the use of a Klett-Summerson photoelectric colorimeter. An aliquot portion of each sample was centrifuged for 30 minutes in a laboratory centrifuge until a clear supernatant liquid was obtained. The clear liquid was carefully decanted and diluted to an appropriate concentration (1°Brix) with distilled water so that light transmittance could be read through a standard red filter (No. 64), which has an approximate spectral range of 600 to 670 millimicrons. Scale reading, proportional to absorbance or optical density was used as an index of color or darkness.

Flavor evaluation and physical appearance through eye appeal of the freshly prepared and stored nectars after dilution was undertaken by a panel of tasters of the Family Nutrition Branch, Medical and Applied Nutrition Division of the Food and Nutrition Research Center, National Institute of Science and Technology. The Hedonic Scale method developed by Peryam and Girardot(15) was used. The following criteria for scoring as to eye appeal (color, attractiveness) and palatability (odor, taste) were used: (9) Like extremely, (8) Like very much, (7) Like moderately, (6) Like slightly, (5) Neither like nor dislike, (4) Dislike slightly, (3) Dislike moderately, (2) Dislike very much, (1) Dislike extremely.

The nectars were diluted with an equal part of water giving a juice with 9° to 10° Brix reading, before presenting to the panel.

### RESULTS AND DISCUSSION

The ascorbic acid content of the mango nectar before fortification was 1 mg per 100 cc of nectar. After fortification

with 50 mg of ascorbic acid per 100 cc and processing, the ascorbic acid content was 48.68 mg per 100 cc showing a loss of 4.6 per cent due to processing. The pH of the freshly processed fortified product was 3 and the total soluble solids was 17° Brix.

Table 1 shows the ascorbic acid content of the stored product and the corresponding percentage retention for a period of 12 months. Retention after 12 months at room temperature was 78.72 per cent while at refrigerated temperature, retention was 85.6 per cent. The ascorbic acid retention decreased as time and temperature of storage increased.

TABLE 1.—*Ascorbic acid content of fortified mango nectars stored at two ranges of temperature and corresponding percentage retention.*

| Length of storage (months) | Refrigerated temperature | | Room temperature | |
|---|---|---|---|---|
| | Ascorbic acid | Retention | Ascorbic acid | Retention |
| | *mg per cent* | *Per cent* | *mg per cent* | *Per cent* |
| 0 | 48.68 | | 48.68 | |
| 2 | 48.44 | 99.51 | 48.07 | 98.75 |
| 4 | 48.09 | 98.79 | 43.42 | 89.19 |
| 6 | 46.93 | 96.40 | 42.70 | 87.72 |
| 8 | 45.25 | 92.95 | 42.66 | 87.63 |
| 10 | 42.81 | 87.94 | 39.56 | 81.26 |
| 12 | 41.67 | 85.60 | 38.32 | 78.72 |

Table 2 shows the effect of storage on the carotene values, acidity and color intensity, expressed as optical density, of fortified mango nectar. The carotene content of the freshly processed product was 0.812 mg per cent. After 12 months at

TABLE 2.—*Effect of storage on the carotene value, color intensity expressed as optical density and acidity of fortified mango nectar.*

| Length of storage (months) | Refrigerated temperature | | | Room temperature | | |
|---|---|---|---|---|---|---|
| | Carotene | Color intensity O. D. | Acidity, per cent anhydrous citric acid | Carotene | Color intensity O. D. | Acidity, per cent anhydrous citric acid |
| | *mg per cent* | | | *mg per cent* | | |
| 0 | 0.812 | 0.327 | 0.45 | 0.812 | 0.327 | 0.45 |
| 2 | 0.560 | 0.378 | 0.44 | 0.517 | 0.400 | 0.44 |
| 4 | 0.121 | 0.320 | 0.43 | 0.072 | 0.284 | 0.47 |
| 6 | 0.130 | 0.282 | 0.44 | 0.146 | 0.292 | 0.46 |
| 8 | 0.126 | 0.285 | 0.44 | 0.046 | 0.297 | 0.44 |
| 10 | 0.109 | 0.332 | 0.45 | 0.170 | 0.334 | 0.46 |
| 12 | 0.202 | 0.306 | 0.47 | 0.217 | 0.324 | 0.47 |

refrigerated storage and room temperature storage, the carotene content was reduced to 0.202 and 0.217 mg per cent, respectively. The carotene values obtained at both storage conditions showed some variations. Similar results were also obtained by other investigators on some products studied.[6, 14] This was attributed to the fact that the chemical determination used measured the collective quantities of several carotene isomers and any isomerism occurring during storage is not detected by the usual chemical determination of carotene. Appreciable losses were noted at both storage conditions studied. Retention at room temperature after 12 months was 27 per cent and at refrigerated temperature, 25 per cent.

Acidity and color intensity of the stored products remained quite stable regardless of time and temperature of storage. The color stability of mango nectar might be compared to that observed in apricot nectar.[20] There was very little change in color noticed in apricot nectar during storage which was attributed to the fact that the carotenoid mixture consists mainly of nonepoxide caratenoids, whereas in peach nectar, where appreciable paling of color on prolonged storage takes place, the carotenoid mixture is composed largely of carotenoid epoxides.

Figures 1 and 2 are graphical representations of the percentage retention of ascorbic acid and carotene on storage respectively.

Mean scores on eye appeal and palatability of the stored products are shown in Table 3. Using the Duncan's New Multiple Range Test, it was found that there was no significant differences at 5 per cent level in eye appeal of the fortified mango nectar stored at room temperature and refrigerated temperature up to 12 months storage. These observations are in conformity with the results of the color intensity tests presented in Table 2. Likewise, there were no significant differences in palatability of the same products during the 12 months storage period. Palatability score, however, at 6 months storage at refrigerated temperature increased slightly although the increase is not significant at 5-per cent level, and remained at that level till the end of the storage study. This might be attributed to the slower rate of deterioration at refrigerated temperature as compared to that at room temperature.
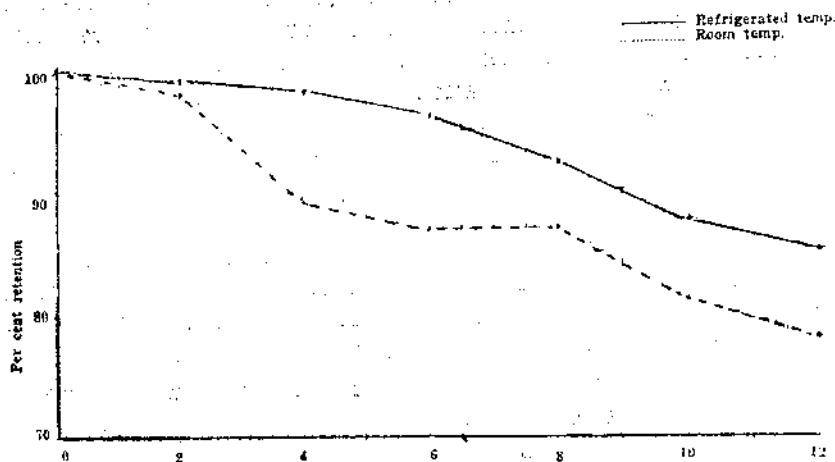
FIG. 1. Percentage retention of ascorbic acid in canned fortified mango nectar on storage at two ranges of temperature.
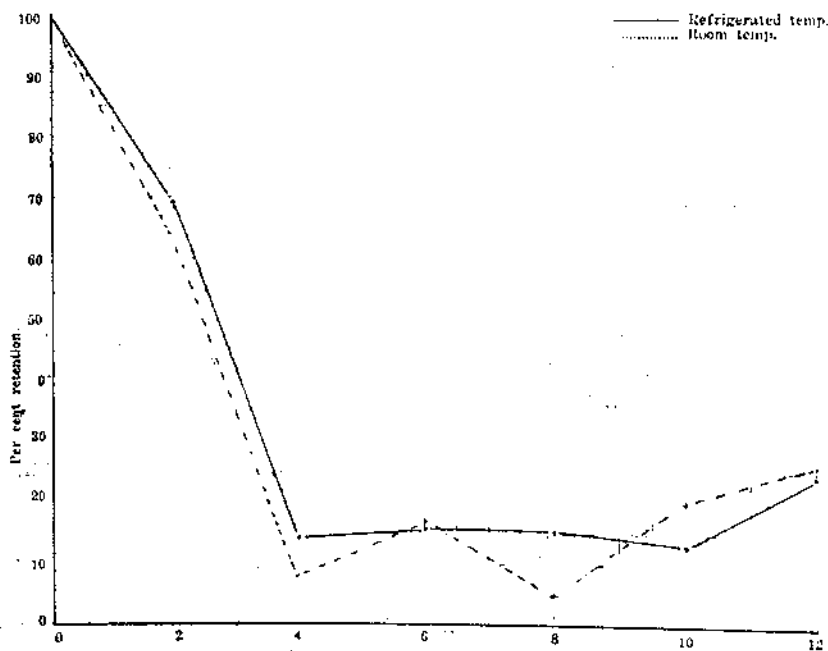


FIG. 2. Percentage retention of carotene in canned fortified mango nectar on storage at two ranges of temperature.

TABLE 3.—*Means scores on eye appeal and palatability of fortified mango nectar stored at two ranges of temperature up to 12 months of storage.*

| Storage period (months) | Eye appeal | | Palatability | |
|---|---|---|---|---|
| | Room temperature | Refrigerated temperature | Room temperature | Refrigerated temperature |
| 0 | 7.4±0.21 | -- | 6.4±0.39 | — |
| 2 | 7.0±0.25 | 7.2±0.21 | 6.3±0.26 | 6.5±0.20 |
| 4 | 7.5±0.23 | 7.7±0.22 | 6.7±0.19 | 6.6±0.38 |
| 6 | 7.2±0.31 | 7.3±0.19 | 6.7±0.31 | 7.3±0.19 |
| 8 | 7.5±0.19 | 7.7±0.19 | 6.8±0.21 | 7.5±0.15 |
| 10 | 7.5±0.23 | 7.4±0.26 | 6.5±0.35 | 7.3±0.28 |
| 12 | 7.2±0.21 | 7.3±0.14 | 6.5±0.45 | 7.2±0.17 |

## SUMMARY AND CONCLUSION

Canned fortified mango (*Mangifera indica* Linn.) nectar was prepared and subjected to storage studies at two ranges of temperature; namely, refrigerated temperature (4° to 5° C) and room temperature (29° to 32° C) for a period of 12 months. Ascorbic acid, equivalent to 50 mg per 100 cc of nectar, was added for fortification.

Ascorbic acid retentions at the end of 12 months at refrigerated and room temperature storage were approximately 86 and 79 per cent, respectively.

There was an appreciable loss of carotene at either temperature range of storage. Retention was approximately 26 per cent after 12 months of storage.

Color intensity and acidity of the stored fortified products were stable throughout the 12 months storage period, regardless of temperature of storage.

Sensory evaluation in terms of eye appeal (color, attractiveness) and palatability (odor, taste) did not change significantly during 12 months storage at both refrigerated and room temperature storage.

members of the Food Research Laboratory, FNRC for their valuable comments on the paper, to Mrs. Erlinda P. Mandap for undertaking the carotene analysis and to the Family Nutrition Branch of the Applied and Medical Nutrition Division of the FNRC, NIST for undertaking the sensory evaluation of the samples.

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# A COMPARATIVE STUDY OF THE STABILITY OF EDIBLE COCONUT OIL PREPARED BY DIFFERENT METHODS

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ONE TEXT FIGURE

The importance of stability in food fats is well accepted. Rancidity in fats not only renders food unpalatable but can also be responsible for the partial destruction of essential fatty acids and vitamins present in the oil.

It is well at the outset to define the terms rancidity and stability as it is meant in this paper. Rancidity is the term employed to indicate an off-flavor in a fat due to either absorption of odors, action of enzymes or atmospheric oxidation. The resistance of a fat to oxidation, i.e., oxidative rancidity, is commonly referred to as its stability. It is expressed as the time required under specified conditions for the fat to become rancid in flavor and odor.

When a sample of oil at an elevated temperature is subjected to a continuous flow of air, oxidation is likely to take place. The first step in fat oxidation is simple addition of oxygen at the double bonds resulting in the formation of peroxides.(2) In later stages, however, these peroxides begin to decompose or react with one another to produce the compounds actually responsible for the rancid flavor and odor. Peroxides, formed during the oxidation of fats can be quantitatively determined and if plotted against time, will give an index of the resistance of an oil to develop rancidity.

Edible oil as produced in the Philippines today is predominantly based on the dry process of manufacture, wherein the oil expressed from the copra (sun-dried or kiln-dried coconut meat) is refined, bleached and deodorized to produce the commercial edible oil now found in the local market. Recently, a wet process of preparing edible oil from the milk extracted from the fresh coconut meat has been developed and tried on a commercial scale. Aside from the question of efficiency and economy of the process, questions arose as to the comparative stability of the oil produced from the two methods or their modifications. This project was therefore conceived in order to determined the stability of commercial and laboratory pro-

cessed edible oils now being produced and to determine which method produces the most stable edible coconut oil.

## EXPERIMENTAL

This study involves the observations of changes in the peroxide value and free fatty acids content of different commercial brands of coconut edible oil and coconut oil produced in the laboratory by the more recently developed modifications of the wet and dry process. Three sets of edible oil samples are included. The first set consists of fresh commercial samples obtained from the various manufacturing firms in and around Manila. The second set consists of laboratory samples prepared by the more recently developed modifications of the wet and dry process. The third set consists of commercial samples obtained from the market, hence not necessarily fresh or of the same age.
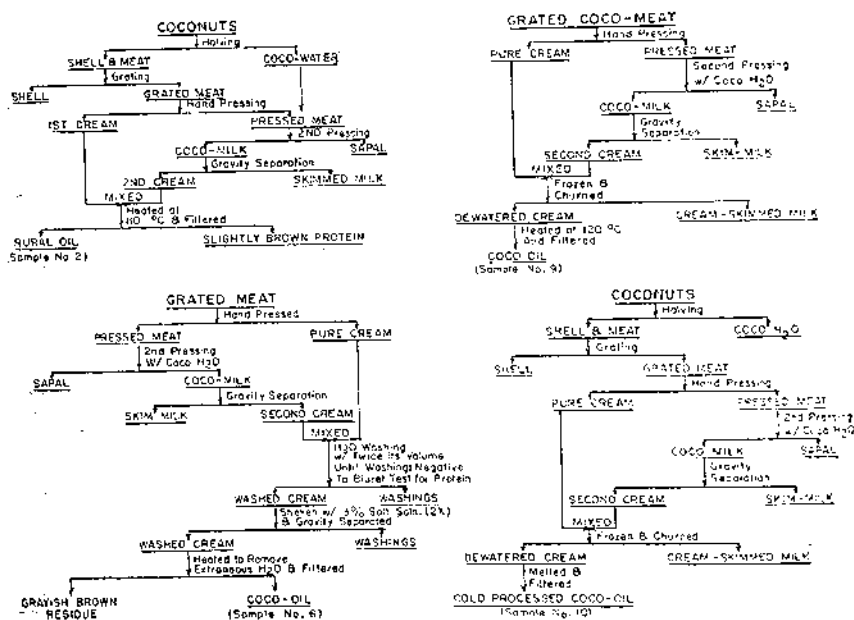


FIG. 1. Variations in the wet processing of coconut oil from fresh coconut meat.

All these samples were subjected to the Fat Stability Tests (AOM) by the American Oil Chemists' Society (AOCS) Official Method, Cd 12–57.(1) This test which is an accelerated method of determining stability essentially consists in bubbling clean air into 25-cc portions of the oil samples maintained at a temperature of 97.7° C. At certain time intervals a small amount of the sample is withdrawn and analyzed for peroxide value. Peroxides are quantitatively estimated by their ability to liberate iodine from potassium iodide in a solution of glacial acetic and chloroform. The peroxide value of an oil is expressed in milliequivalents of oxygen per 1,000 grams of oil. AOCS Official Method, Cd 8–53 for peroxide value determination was used in these experiments. As soon as peroxide values in the range of 75 to 125 milliequivalents were obtained, the test for that particular sample was stopped and the peroxide values were plotted on rectangular coordinate paper against the number of hours of aeration. In this paper, AOM (Active Oxygen Method) stability is defined as the number of hours required for a sample of oil to develop a peroxide value of 100 milliequivalents under the specific conditions of the test.

The free-fatty acids content of all the samples were determined before and after the stability tests.

<center>RESULTS AND DISCUSSIONS</center>

Table 1 presents the comparative (AOM) stabilities of fresh edible oil samples obtained from commercial processing plants. It may be observed from this table that the commercial edible oil samples processed from copra gave relatively higher stabilities than the samples prepared direct from the fresh meat by the wet process.

In the second set of experiments, results of which are presented in Table 2, the samples used were all prepared in the laboratory just before the AOM tests were conducted. It is interesting to note in this table that all the samples registered very high stabilities regardless of whether they were prepared from the fresh meat or from the dried meat.

Table 3 gives the stabilities of edible oil samples picked at random from the market. Except for one sample, all the others had very short stabilities indicating that the samples were no longer fresh. It has also been observed that the free fatty acids content of the oil did not change appreciably as the oils developed high peroxide values.

TABLE 1.

| Sample number | Description of sample | AOM stability, [1] (hours) | Original FFA (as oleic) | Final FFA (as oleic) |
|---|---|---|---|---|
| | | | Per cent | Per cent |
| 21 ⎫ 20 ⎭ | Freshly prepared samples from a commercial plant processing edible coconut oil direct from the fresh meat | 19.8 20.5 | 0.08 0.07 | 0.37 0.15 |
| 22D ⎫ 13 ⎬ 15 ⎭ | Freshly prepared samples from commercial plants processing edible oil from copra | 110.8 105.3 143.5 | 0.05 0.06 0.02 | 0.21 1.22 0.31 |

TABLE 2.

| | | | | |
|---|---|---|---|---|
| 6 ⎫ 9 ⎬ 2 ⎪ 10 ⎭ | Sample of oil prepared by the wet process developed in the laboratory | 173.5 330.5 185.5 264.5 | 0.13 0.11 0.06 0.06 | 0.27 0.15 0.19 |
| 33 | Oil expressed from commercial desiccated coconut | 307.5 | 0.03 | 0.26 |
| 29 | Oil expressed from coconut meat dried in a fluidized bed | 222.2 | 0.06 | 0.79 |

TABLE 3.

| | | | | |
|---|---|---|---|---|
| 20 ⎫ 21 ⎬ 35 ⎪ 13 ⎭ | Commercial samples picked at random from the market | 169.8 25.8 59.2 33.0 | 0.04 0.08 0.06 0.06 | 0.21 0.62 0.23 0.29 |

[1] Average of at least six determinations.

On account of the observed differences in stability of samples in Tables 1 and 2 it becomes important to include in this discussion a general idea of the major differences in the methods of preparation of these samples.

Samples 20 and 21 came from a commercial plant processing edible oils by extracting the milk from fresh grated coconut meat, separating the cream by centrifugation, freezing the cream to break-up the oil in water emulsion and subsequently warming the frozen cream to separate oil insoluble material from the clear oil which is then deodorized, prior to packing into tins ready for the consumer.

Samples 22D, 13 and 15 came from commercial plants processing edible oil by expressing the crude oil from copra. The crude coconut oil is then subjected to bleaching, alkali refining and deodorization processes before they are finally packed for the market.

Samples 6, 9, 2 and 10 were all prepared in the laboratory of Dr. Dionisio M. Birosel following the schemes shown in Fig. 1.

Sample 33 was expressed from a desiccated coconut product manufactured by a commercial plant in Laguna Province. The expressed oil was filtered and used subsequently in the tests.

Sample 29 was expressed from granulated coconut. Granulated coconut is the product obtained when fresh white coconut meat is comminuted to colloidal size and dried in a Fluidized Bed Dryer to a moisture content of about 2 per cent.

After a thorough study of the methods by which the different edible oil samples were prepared, it is interesting to note that while in Table 1 edible coconut oil processed from copra appeared to have better stability than oil prepared from "gata" cream (coconut oil in $H_2O$ emulsion) expressed from the fresh meat, such is not the case in Table 2 where it is shown that oil prepared from the cream of the fresh meat registered high AOM stabilities ranging from 126 to 330 hours. This might have been due to some unforseen faults in the commercial scale processing of oil from the cream of the fresh meat which is no longer within the scope of the present study. Similarly, high values were also obtained for oils expressed from the desiccated coconut and from the granulated coconut.

Another important observation is the fact that stability values were comparatively higher for all the oils that did not undergo any alkali-refining, bleaching and deodorization processes.

Results and observations gathered in this study have led to the possibility that certain substances (natural antioxidant) inherent in the oil and responsible for its resistance to oxidative rancidity are removed in either the bleaching, akali-refining, or deodorization processes.

This study has given rise to another problem: that of determining the antioxidants present in the oil as it undergoes various stages of processing.

### SUMMARY

The AOM stabilities of edible coconut oil samples prepared by various methods were determined. Results obtained showed that the stability of oil samples which underwent alkali-refining, bleaching and deodorizing processes were lower than that of oils that were not treated. This observation has led to the possibility that certain substances (natural antioxidants) inherent in the oil and responsible for its resistance to oxidative rancidity are removed in either of the aforementioned processes.

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# SCREENING OF PHILIPPINE PLANTS FOR ANTI-CANCER ACTIVITY

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In a previous article, Masiluñgan, et al.(3) reported the results of the anticancer activity of medicinal plants locally used in the treatment of cancer. Due to the encouraging results obtained in the above study the authors screened other higher plants for anticancer activity using the Ehrlich ascites tumor cells.

## MATERIALS AND METHODS

Plants around the vicinity of the National Institute of Science and Technology and other plants collected by the authors during their field trips were used in this investigation. Extracts were prepared from the different parts of the plants while they were still fresh and, when extraction could not be done promptly, the specimens were kept in a refrigerator to maintain their freshness.

The procedure followed for collecting and sampling of plant materials and in preparing the extract was as described in a previous paper by Masiluñgan, et al.(2) The anticancer activity of the crude extracts was determined by following the procedure of Yamasaki, et al.(5) with some modification.

The crude extract was prepared by macerating 5 gm of a sample in 20 cc of solvent for 24 hours and then filtered.

Ethyl alcohol, methyl alcohol, ether, petroleum ether, acetone, 1-per cent solution of acetic acid and 5-per cent sodium bicarbonate, 1-per cent solution of hydrochloric acid and distilled water were the solvents used for extraction.

The Ehrlich ascites tumor cells (EATC) used in this study were obtained through the courtesy of Dr. Serafin Juliano, head of the Department of Pathology, Institute of Medicine, Far Eastern University. The stock culture was carried in "Strong A" mice by transplanting intraperitoneally 0.2 cc of ascites fluid containing around 2 million 7-day-old EATC.

On the seventh day, the mice were killed and EATC were withdrawn from the peritoneum by sterile needle and syringe. They were placed in sterile calibrated centrifuge tubes and were centrifuged for about 3 minutes at 1,000 rpm. The sedimented tumor cells were washed twice with about an equal volume of phosphate buffer solution of pH 7.1, and then adjusted, such that the suspension contained 5,000,000 cells per cc. The buffer solution is prepared by dissolving 16-gm NaCl, 0.4-gm KCl. 2.3-gm $Na_2HPO4$, and 0.4-gm $KH_2PO4$ in distilled water to make 2,000-cc solution and then sterilized. Cells to be used for testing should not be more than 1 hour after they have been withdrawn from the mice. Bloody ascites tumor cells can also be used with the same efficiency as those free from blood provided that the red blood cells are first removed. One way of removing the red cells is by hemolyzing the red cells with ice-cold sterile distilled water for 30 seconds and then immediately adding ice-cold 3½-per cent sodium chloride solution to make the final concentration almost like that of physiological salt solution. It is centrifuged at 1,000 rpm for 3 minutes. The supernatant liquid is removed, the tumor cells washed once with the buffered solution and then adjusted to the desired concentration as above.

The test plate was prepared by pouring 5 cc of phosphate buffer solution containing 5-million tumor cells per cc into a sterile 90-mm diameter petri dish. Then 5 cc of melted Hank's agar cooled to 43° C was poured into the dish, mixed thoroughly and then allowed to solidify on a level surface for one hour.

Filter paper discs, saturated with the plant extracts were carefully placed on the seeded agar. For control, a disc saturated with corresponding solvents and another disc saturated with mercuric chloride solution (1:1,000) which served as positive control, were also placed on the seeded agar. This was replicated twice.

The test plate was incubated at room temperature for 18 hours. The disc were removed and the surface of the seeded agar washed with sterile distilled water and then covered with 1 to 1 mixture of 5-per cent plasma solution and 0.4-per cent solution of methylene blue as redox agent for 15 seconds. Flat glass disc was gently placed inside the petri dish to cover the media. The excess dye was poured off. The plate was further incubated for 3 hours at room temperature and then

examined for unreduced dye. The diameter of the unreduced dye was measured in millimeter.

## RESULTS AND DISCUSSIONS

Since our last report[3] on the anticancer activity of Philippine medicinal plants used in the treatment of cancer, 1,179 extracts from different parts of 120 species of plants belonging to 58 families were screened for anticancer activity.

After testing 108 extracts prepared from 12 samples of 11 species of plants using 25-million EATC per dish plate, it was found out that 92 per cent of the samples examined were positive for anticancer activity. In order to narrow down the selection of potential anticancer agents, 25- and 100-million cells per dish plate were used to test 135 extracts from 15 samples of 12 species of plants. As shown in Table 1, 87 per cent of the samples were found positive for anticancer activity when 25-million cells were used. But when the number of EATC was increased to 100-million cells, the number of positives from the same samples was reduced to 47 per cent. Hence, as a modification of Yamasaki's procedure, 1 cc of phosphate buffer solution containing 100-million EATC mixed with 9 cc of Hank's agar was used in the screening of plants for anticancer activity.

Using the following solvents: distilled water, 1-per cent hydrochloric acid, 1-per cent acetic acid, 5-per cent sodium bicarbonate solution, ether, methanol, 95-per cent ethanol, acetone and petroleum ether, 1,179 extracts from 131 samples of 120 species of plants were prepared and tested for anticancer activity. The results are shown in Table 2. Thirty six per cent of the samples tested showed anticancer activity. Of the 132 samples of plants, 11 yielded anticancer activity of varied potency with the use of distilled water as solvent; 23 with 1-per cent acetic acid; 16- to 1-per cent hydrochloric acid; 17 with 5-per cent sodium bicarbonate solution; 18 with ether; 20 with methanol; 15 with ethanol; 12 with petroleum ether; and 9 with acetone.

The positive results depended on the capability of the crude extracts to inactivate the tumor cells. Dehydrogenases present in the normal tumor cells, activate the hydrogen present in the molecule of the metabolite during biological oxidation. Under anaerobic condition the released hydrogen combines with

TABLE 1.—*Plants tested for anticancer activity using 25,000,000 cells/cc as well as 100,000,000 cells/cc.*

| Plant | | Number of tumor cells | Relative inhibitive activity * of extracts in different solvents and zones of inhibition of Ehrlich ascites tumor cells in millimeters. | | | | | | | | | Showing inhibitive activity of HgCl₂ ** (1:1,000) as positive control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scientific name | Part used | | Water | Acetic acid 1 per cent | Hydrochloric acid 1 per cent | Sodium bicarbonate 5 per cent | Ether | Methanol | Ethanol | Acetone | Pet. ether | |
| *Acalypha wilkesiana* Muell.-Arg... | Leaves | 25M/cc | -- | +++ 12 | + 7 | . | - - | -- 7 | + 6 | + 7 | - - | ++++ 8 |
| | | 100M/cc | - - | . . | . . | -- | . . | . . | . . | . . | --- | ++++ 7 |
| *Lochnera rosea* Reichb. (chichirica) | Leaves | 25M/cc | + 7 | + 8 | - - | -- | +++++ 8 | + 8 | ++++ 12 | + 5 | + 3 | +++++ 9 |
| | | 100M/cc | --- | . . | - - | . . | . | . | -- | . . . | -- | ++++ 7 |
| *Hibiscus rosasinensis* (gumamela) | Leaves | 25M/cc | --- | . . | . | -- 12 | . | . | -- | ++ 9 | — | ++++ 9 |
| | | 100M/cc | - - | | - - | | | | --- | ++++ 15 | - - | ++++ 8 |
| | Flowers | 25M/cc | --- | . . | . | -- 15 | | | - - | +++ 11 | - - | ++++ 9 |
| | | 100M/cc | ++ 16 | +-- 11 | - - | | | | | | --- | ++++ 8 |
| *Nothopanax crispatum* | Leaves | 25M/cc | ++ 17 | +-- 10 | . 15 | . | ++++ 12 | ++++ 13 | ++++ 11 | +++++ 12 | --- | ++++ 11 |
| | | 100M/cc | --- | -- 11 | . | | | | | . | - - | ++++ 8 |
| | Stems | 25M/cc | ++++ 12 | . 20 | -- 20 | -- 20 | | | -- 11 | ++++ 15 | . . | ++++ 11 |
| | | 100M/cc | . . | . 16 | | | | | | | - - | ++++ 8 |
| *Gliricidia sepium* (Jacq.) Steud. (Kaukawate) | Leaves | 25M/cc | . | +-- 8 | | | | -- 6 | -- 7 | | - - | +++++ 10 |
| | | 100M/cc | | | | | | | | | | +++++ 7 |
| | Flowers | 25M/cc | . | | - | | | | | | - - | +++++ 4 |
| | | 100M/cc | | | | | | | | | | +++++ 7 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Carica papaya | Leaves | 25M/cc | +++ 11 | + 8 | + 10 | — | — | ++++ 11 | ++++ 12 | +++ 7 | — | ++++ 12 |
| | | 100M/cc | — | | | | | | | | | +++- 9 |
| Sandoricum koetjape (santol) | Leaves | 25M/cc | — | + 7 | + 7 | + 6 | +++ 8 | ++++ 6 | + 6 | ++++ 7 | + 6 | ++++ 10 |
| | | 100M/cc | — | | | | | | | | | +++ 10 |
| Euphorbia pulcherrima (poinsettia) | Leaves | 25M/cc | — | — | — | — | ++++ 7 | — | — | ++++ 9 | — | ++++ 8 |
| | | 100M/cc | — | ++ 11 | — | ++ 12 | | — | — | | | +++ 8 |
| Lagerstroemia speciosa (Linn.) Pers. (banaba) | Leaves | 25M/cc | — | — | + 9 | — | ++ 8 | ++ 7 | +++ 6 | +++ 7 | + 4 | ++++ 8 |
| | | 100M/cc | — | — | — | — | | | | | | +++ 7 |
| Chrysophyllum cainito (starapple) | | 25M/cc | + 5 | + 6 | | — | ++++ 12 | +++ 7 | ++++ 7 | +++ 7 | ++ 4 | ++++ 8 |
| Ipomaea batatas | | 100M/cc | — | — | ++ 7 | | | ++ 6 | ++ 6 | +++ 6 | — | +++ 10 |
| (camote) | Leaves | 25M/cc | — | — | — | — | — | ++++ 11 | — | — | — | ++++ 16 |
| | | 100M/cc | — | — | — | — | | | | | +++ 9 | +++ 9 |
| Dioscorea hispida | Leaves | 25M/cc | — | — | — | — | — | — | — | — | — | +++ 9 |
| | | 100M/cc | — | — | — | — | | — | — | — | | +++ 7 |

Activity of solvents used as extractants

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Controls | | | — | — | — | — | | — | — | — | — | — |

* Meaning of Symbols: +++, for marked inhibition; ++, for moderate inhibition; +, for slight inhibition; —, absence of anticancer activity. The number refers to the diameter of unreduced dye in millimeters.

** Mercuric chloride aqueous solution (1:1000) which is known to markedly inhibit the ascites tumor cells in the experiment of DiPaolo(1) and Miyamura(6) was used as control to show evidence of validity of the approach.

the methylene blue used as redox agents to form the leuco methylene compounds which is colorless.

The presence of circular blue zone at the site where formerly the filter paper, wet with the plant extract was located indicates positive result. It suggests that the test extracts exert an inhibitory effect on the activity of the cells.

The above tests are qualitative and do not at all portray the exact reaction of the active agents against the tumor cells, but they are necessary in the preliminary search for plants with potential anticancer activity for further study.

## SUMMARY

1. One thousand one hundred seventy-nine extracts from different parts of 120 species of plants belonging to 58 families were screened for anticancer activity or Ehrlich ascites tumor cells by the filter paper disc method. Thirty six per cent of the samples tested showed anticancer activity.

2. Anticancer agents of varied potency were extracted by one or more of the nine solvents used.

3. Of the 131 samples of different parts of 120 species of plants, 11 yielded anticancer activity with distilled water as extractant; 24 with 1-per cent acetic acid; 16 with 1-per cent hydrochloric acid, 17 with 5-per cent solution of sodium bicarbonate; 18 with ether; 20 with methanol; 15 with ethanol; 12 with petroleum ether; and 9 with acetone.

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TABLE 2.—*Anticancer activity of extracts from plants.*

| Plant [**] | | | Relative inhibitive activity [*] of extracts in different solvents and zones of inhibition of Ehrlich ascites tumor cells in millimeters. | | | | | | | | | Showing inhibitive activity of HgCl₂ (1:1,000) as positive control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scientific test | Local name | Part examined | Water | Acetic acid 1 per cent | Hydrochloric acid 1 per cent | Sodium bicarbonate 5 per cent | Ether | Methanol | Ethanol | Petroleum ether | Petroleum Acetone | |
| Pteridaceæ | | | | | | | | | | | | |
| Pteris aquilinum | | Leaves | — | — | — | — | — | — | — | — | — | + + + 8 |
| Cycadaceæ | | | | | | | | | | | | |
| Cycas rumphii Miq. | Pitogo | Leaves | — | — | — | — | — | — | — | — | — | + + + + 8 |
| Pinaceæ | | | | | | | | | | | | |
| Pinus insularis | Baguio pine | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Pandanaceæ | | | | | | | | | | | | |
| Pandanus luzonensis Merr. | Pandan-luzon | Roots | — | — | — | — | — | — | — | — | — | + + 10 |
| Hydrocharitaceæ | | | | | | | | | | | | |
| Hydrilla verticellata | | Whole plant | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Vallisneria gigantea Graebn. | Sintas-sintasan | Whole plant | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Gramineæ | | | | | | | | | | | | |
| Imperata cylindrica (L.) Beauv. var. koenigii | Cogon | Leaves | — | — | — | — | — | — | — | — | — | + + + + 10 |
| Zea mays Linn. | Mais | Leaves | — | + + + + 10 | + + + 7 | — | — | — | — | — | — | + + + + 7 |
| Palmæ | | | | | | | | | | | | |
| Livistona rotundifolia | Anahaw | Leaves | — | — | — | — | — | — | — | — | — | + + + + 10 |
| Araceæ | | | | | | | | | | | | |
| Epipremnum Merrillii Engl. and Krause | Talahib | Leaves | — | + + + 8 | — | + + + 8 | — | — | — | — | — | + + + + 10 |
| Aglaonema minus Hook. F. | | Leaves | — | — | — | — | — | — | — | — | — | + + + + 9 |
| Caladium bicolor Vent. | Gabi | Leaves | — | — | — | — | — | — | — | — | — | + + + + 9 |
| Commelinaceæ | | | | | | | | | | | | |
| Commelina benghalensis Linn. | Alibangbang | Leaves | — | — | — | — | — | — | — | — | — | + + + + 9 |
| Pontederiaceæ | | | | | | | | | | | | |
| Eichornea crassipes (Mart) Solms. | Water hyacinth | Whole plant | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Amaryllidaceæ | | | | | | | | | | | | |
| Amaryllis belladona | | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Zephyranthes rosea Lindl. | Kulog | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| | | Bulbs | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Dioscoreaceæ | | | | | | | | | | | | |
| Dioscorea hispida Dennst. | Nami | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| | | Bulbs | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Iridaceæ | | | | | | | | | | | | |
| Belamcanda chinensis (Linn.) DC. | Abaniko | Leaves | — | — | — | — | — | — | — | — | — | + + 7 |
| Musaceæ | | | | | | | | | | | | |
| Heliconia psittacorum Linn. | Bird of paradise | Leaves | — | — | — | — | — | — | — | — | — | + + + + 9 |
| Musa sapientum Linn. | Saging | Leaves | — | — | — | — | — | — | — | — | — | + + + + 8 |
| Marantaceæ | | | | | | | | | | | | |
| Maranta arundinacea Blanco | Aroru | Leaves | — | — | — | + + + 11 | — | — | — | — | + 7 | + + 6 |
| Casuarinaceæ | | | | | | | | | | | | |
| Casuarina equisetifolia Linn. | Agoho | Leaves | — | + + + + 5 | — | + + + + 6 | — | — | — | — | — | + + + + 9 |
| Piperaceæ | | | | | | | | | | | | |
| Peperomia pellucida (L.) HBK. | Ulasiman-bato | Leaves | — | — | — | — | — | — | — | — | + + + + 9 | + + + + 8 |
| Moraceæ | | | | | | | | | | | | |
| Ficus nota (Blanco) Merr. | Tibeg | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Loranthaceæ | | | | | | | | | | | | |
| Scurrula parasitica | Dapongkahoi | Leaves | — | — | — | — | — | — | — | — | — | + + 6 |
| Amaranthaceæ | | | | | | | | | | | | |
| Amaranthus spinosus Linn. | Urai | Leaves | — | — | — | — | — | — | — | — | — | + + + + 8 |
| Gomphrena globosa | Bachelor's buttons | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Nyctaginaceæ | | | | | | | | | | | | |
| Bougainvillaea spectabilis Willd. | Bougainvilla | Leaves | — | + + + + 13 | — | + 5 | + 6 | + 6 | + 6 | — | — | + + + + 10 |
| Portulacaceæ | | | | | | | | | | | | |
| Corculum leptopus (Hook and Arn) Stuntz | Cadena de amor | Leaves | — | — | — | — | — | — | — | — | — | + + + + 8 |
| Portulaca oleracea Linn. | Olasiman | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Talinum triangulare | Talinum | Leaves | — | — | — | — | — | — | — | — | — | + + 7 |
| Menispermaceæ | | | | | | | | | | | | |
| Arcangelisia flava (Linn.) Merr. | Lagtang | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |
| Anonaceæ | | | | | | | | | | | | |
| Anona squamosa Linn. | Atis | Leaves | — | + + + + 9 | + + + + 6 | + 8 | + 10 | + 11 | + 9 | + 7 | + + 5 | + + + + 7 |
| Cananga odorata (Lam.) Hook. | Ilang-ilang | Leaves | — | — | — | — | — | — | — | — | — | + + + + 8 |
| Lauraceæ | | | | | | | | | | | | |
| Persea americana Mill. | Avocado | Leaves | + + + 15 | + + + 12 | + + 8 | — | — | — | — | — | — | + + + + 10 |
| Moringaceæ | | | | | | | | | | | | |
| Moringa oleifera Lam. | Malungai | Leaves | — | — | — | — | — | — | — | — | — | + + + + 7 |

TABLE 2.—*Anticancer activity of extracts from plants*—Continued.

| Plant *** | | | Relative inhibitive activity* of extracts in different solvents and zones of inhibition of Ehrlich ascites tumor cells in millimeters | | | | | | | | | Showing inhibivitive activity of HgCl** (1:1,000) as positive control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Scientific test | Local name | Part examined | Water | Acetic acid 1 per cent | Hydrochloric acid 1 per cent | Sodium bicarbonate 5 per cent | Ether | Methanol | Ethanol | Petroleum ether | Petroleum acetone | |
| *Polyscias crispatum* (Bull.) Merr. | | Leaves | — | — | — | — | — | — | .... | — | .. | + + + 7 |
| *Polyscias guilfoylei* Bailey | | Leaves | — | — | — | .. | — | — | ... | .. | — | + + + + 7 |
| Sapotaceæ | | | | | | | | | | | | |
| *Chrysophyllum cainito* Linn. | Starapple | Leaves | — | — | + + 7 | — | — | + + 6 | + + 6 | + + 6 | . . | + + + + 10 |
| Oleaceæ | | | | | | | | | | | | |
| *Jasminum grandiflorum* Linn. | Jasmin | Leaves | — | . . | — | — | — | .... | . . | — | — | + + + + 10 |
| | | Stem | — | — | — | — | — | . . | .... | — | — | + + + + 10 |
| Apocynaceæ | | | | | | | | | | | | |
| *Allamanda cathartica* Linn. | Kampanero | Leaves | — | — | — | + + + + 11 | + + 6 | + 6 | — | — | — | + + + 8 |
| *Alstonia scolaris* (L) R. Br. | Dita | Leaves | — | — | — | — | .... | — | — | — | — | + + + + 7 |
| *Lochnera rosea* (Linn.) Reichb. | Chichirica | Leaves | — | — | — | — | — | . . | . . | — | — | + + + + 7 |
| | | Stem | — | — | — | — | — | — | — | — | . . | + + + + 7 |
| | | Flowers | — | — | — | — | — | — | — | — | . . | + + + + 7 |
| *Plumiera acuminata* Ait. | Kalachuchi | Leaves | . . | — | — | — | — | — | — | — | ... | + + + + 7 |
| *Tabernaemontana pandacaqui* Poir. | Pandakaki | Leaves | — | — | — | — | — | . . | — | — | .... | + + + + 9 |
| *Therecia peruviana* (Pers.) Merr. | Campanilla | Leaves | — | . . | + + + 10 | — | — | + + + 8 | + + + 10 | — | + + 1 | + + + + 10 |
| Convolvulaceæ | | | | | | | | | | | | |
| *Ipomoea batatas* (Linn.) Poir. | Kamote | Leaves | — | — | — | — | — | — | .... | — | — | + + + + 9 |
| Boraginaceæ | | | | | | | | | | | | |
| *Ehretia microphylla* Linn. | Alañgitñgit | Leaves | — | — | . . | — | — | — | . . | — | . . | + + 7 |
| *Heliotropium indicum* Linn. | Buntot-leon | Leaves | + + 11 | — | — | — | — | — | — | — | . . | + + 8 |
| Verbenaceæ | | | | | | | | | | | | |
| *Clerodendron intermedium* Cham. | Larvan-anito | Leaves | + + 12 | — | — | — | — | . . | . . | — | — | + + 7 |
| *Clerodendron thomsonae* Ball. | Secreto de amor | Leaves | — | . . | . . | — | . . | . . | — | — | .... | + + + + 7 |
| *Holmskioldia sanguinea* | | Leaves | + + 8 | + + 7 | — | — | — | .... | .... | — | — | + + 7 |
| *Premna odorata* Blanco | Alagaw | Leaves | — | — | — | — | — | .... | — | . . | — | + + + + 10 |
| *Tectona grandis* Linn. | Tekla | Leaves | — | — | + + 4 | — | — | — | — | . . | . . | + + + + 6 |
| *Vitex aherniana* Merr. | | Leaves | — | — | + + 4 | — | — | — | — | — | — | + + + + 5 |
| *Vitex negundo* Linn. | Lagundi | Leaves | . . | + 3 | . . | — | + 3 | + 5 | + 7 | + 3 | . . | + + + + 7 |
| | | Flowers | — | — | — | — | + + 8 | — | — | + 6 | . . | + + 10 |
| Labiatæ | | | | | | | | | | | | |
| *Coleus blumei* Benth. | Mayana | Leaves | — | — | — | — | . . | . . | — | — | — | + + + + 7 |
| *Leucas lavandulifolia* Sm. | Solasolasihan | Whole plant | — | — | — | — | — | . . | — | — | — | + + + + 7 |
| Solanaceæ | | | | | | | | | | | | |
| *Cestrum nocturnum* Linn. | Dama de noche | Leaves | + 6 | — | — | — | — | + 5 | + 5 | + 5 | — | + + 6 |
| *Datura metel* Linn. | Talong-punai | Leaves | — | — | — | — | — | — | . . | — | . . | + + + + 7 |
| *Solanum melongena* Linn. | Talong | Leaves | . . | — | + + + 5 | + 4 | + + + 7 | — | — | — | — | + + + + 8 |
| Scrophulariaceæ | | | | | | | | | | | | |
| *Scoparia dulcis* Linn. | Sampalokan | Leaves | — | — | — | — | + + 9 | + + 7 | — | — | — | + + 8 |
| Acanthaceæ | | | | | | | | | | | | |
| *Justicia gendarussa* Burm. f. | Limangsugat | Leaves | . . | — | — | — | . . | — | .... | — | . . | + + 7 |
| *Odontonema callistachyum* | | Leaves | . . | .... | — | . . | . . | — | — | — | — | + + + + 7 |
| Rubiaceæ | | | | | | | | | | | | |
| *Borreria laevis* | | Whole plant | .... | — | — | . . | — | — | — | — | — | + + + + 7 |
| *Gardenia augusta* (Linn.) Merr. | Rosal | Leaves | .... | — | — | . . | . . | — | — | — | — | + + + + 7 |
| *Ixora finlaysoniana* | Santan puti | Leaves | + + + + 9 | + + + + 11 | — | — | + + + + 7 | . . | — | — | + + + 7 | + + + 9 |
| *Ixora macrothyrsa* | Santan pula | Leaves | + + 10 | + + 5 | — | — | . . | . . | — | — | — | + + 8 |
| Compositæ | | | | | | | | | | | | |
| *Ageratum conyzoides* Linn. | Bulak manok | Leaves | . . | . . | . . | .... | .... | .... | .... | — | — | + + + + 8 |
| *Cosmos caudatus* HBK | Cosmos | Leaves | . . | — | . . | . . | — | .... | — | + 7 | + + 7 | + + + + 8 |
| *Cirsium luconiense* Merr. | | Leaves | . . | + + + + 10 | + + + + 10 | + + + 10 | + + + + 17 | + + + + 12 | + + + + 9 | + + 7 | + + 7 | + + + + 10 |
| *Eclipta alba* (Linn.) Hassk. | Higis manok | Leaves | . . | — | — | + + + 13 | + + + 7 | — | — | . . | .... | + + + + 7 |
| *Emilia sonchifolia* (Linn.) DC. | Lagulinau | Leaves | . . | — | — | . . | — | — | — | . . | .... | + + + + 5 |
| *Eupatorium adenophorum* Spreng. | | Leaves | .... | — | .... | — | — | .... | — | — | — | + + + + 7 |
| *Gynura aurantiaca* | Wondering Jew | Leaves | . . | — | — | — | — | — | — | . . | — | + + + + 10 |
| *Gynura crepidioides* | | Whole plant | — | . . | — | . . | .... | — | — | . . | — | + + + + 5 |
| *Sonchus arvensis* Linn. | Lamlampaka | Leaves | — | — | — | — | .... | — | — | . . | — | + + + + 7 |
| *Tagetes erecta* Linn. | Amarillo | Leaves | . . | + + + + 12 | + + + + 9 | . . | — | — | — | — | — | + + + + 10 |
| *Vernonia cinerea* (Linn.) Less. | Tagulinai | Leaves | . . | | | | | | | — | — | — |
| | | | | Activity of solvents used as extractants | | | | | | | | |
| Controls | | | — | — | — | — | — | — | — | — | — | — |

* Meaning of Symbols: + + +, for marked inhibition; + +, for moderate inhibition; for slight inhibition; —, absence of anticancer activity. The number refers to the diameter of unreduced dye in millimeters.

** Mercuric chloride aqueous solution (1:1000) which is known to markedly inhibit the ascites tumors cells in the experiment of DiPaolo (1) and Miyamura (6) was used as control to show evidence of validity of the approach.

*** The system of classification is patterned after that of Engler and Prantl.

# THE ALKALOIDS OF ANONA MURICATA LINN.*

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TWO PLATES AND ONE TEXT FIGURE

The Anonaceæ is perhaps one of the more represented families in the Philippines. Although some are endemic, many were brought by the Spaniards from South America and Mexico. Belonging to the latter groups are members of the genus *Anona* Linn., which are chiefly cultivated throughout the country for their delicious fruits. Santos and Reyes (9) isolated anonaine (I) from *Anona squamosa* (Tag. "atis") and from the bark of *Anona reticulata* (Tag. "anonas").(10) Gopinath and Govindachari (4) in a later investigation of the root-bark of *A. reticulata* L. obtained a phenolic base, reticuline (II) together with anonaine. In 1941, Meyer (7) reported the presence of
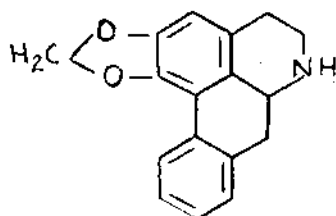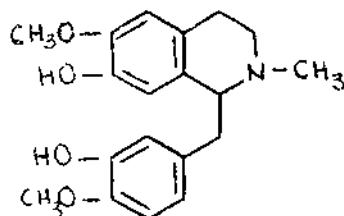


I                                                                     II

two alkaloids in the bark of *Anona muricata* Linn. (Tag. "guayabano") and gave the names muricinine and muricine for the phenolic and nonphenolic bases, respectively. In spite of

the many years that have elapsed, the chemical structures of these alkaloids have not been clarified. We are now reporting on the further studies these tertiary bases from *Anona muricata* Linn.

In this present report, we have developed a method of isolating the alkaloids, described in the experimental part. The method facilitated the isolation of one phenolic and two nonphenolic alkaloids.

The perchlorate salt of the phenolic base melted at 206–207° which corresponds to the melting point of muricinine perchlorate isolated by Meyer. It gives the same green color with ferric chloride. It gives a greenish blue color with concentrated sulfuric acid in the cold, turning blue to violet upon heating to 130°. It turns pink with Millon's reagent. Gopinath and Govindachari observed the same characteristic color reactions for reticuline perchlorate. The phenolic base (muricinine) gave $[\alpha]_D^{33} = +70°$ ($c = 1$, ethanol). The elemental analysis, and the molecular weight, methoxyl and methylimide determinations lead to the molecular formula $C_{19}H_{23}O_4N$ which closely agreed with those reported for reticuline. The UV spectrum gave $\lambda$ max (EtOH) 283 m$\mu$ (log $\varepsilon$ 3.72) and $\lambda$ max (EtOH) 254 m$\mu$ (log $\varepsilon$ 2.78) which is the characteristic absorption pattern of benzylisoquinoline bases. The IR spectrum indicates the presence of bonded hydroxyl groups (3300 cm $^{-1}$, vs.) and is general in agreement with the IR spectrum reported by Kunitomo[6] for reticuline. To elucidate further the structure of the isolated phenolic base, its mass spectra was measured. The molecular ion peak M+ is not registered, instead two characteristic peaks were observed: the base peak at m/e 192 and the peak at m/e 177. According to Djerassi and co-workers[8] the mass ion M+ of the benzyliso-quinoline bases is not observed but two mass ions are registered corresponding to the isoquinoline portion of the molecule and to the fragment formed after the cleavage of a methyl radical from the base peak. On the basis of these observations, the following fragmentation is rationalized for muricinine: the base peak at m/e 192 formed by fission of the benzylic bond beta to the nitrogen atom. Another important ion is at m/e 137 which corresponds to the benzyl portion of the benzylisoquinoline molecule. The above data established the fact that muricinine is identical with reticuline.
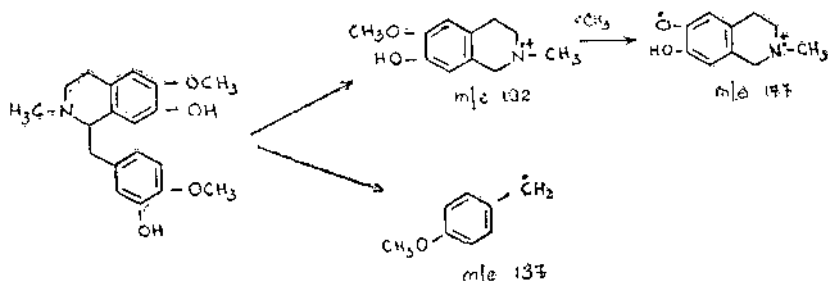
The nonphenolic fraction contains two alkaloids, however, only one was obtained in such an amount to warrant chemical and physical methods of analysis. The nonphenolic bases were separated by column chromatography on neutral alumina. No hydrobromide salt could be prepared crystalline from the two bases and therefore no relationship with the nonphenolic base isolated by Meyer could be established.

The nonphenolic base A was obtained as an oily residue from ether and would not crystallize except as its perchlorate salt from 50-per cent ethyl alcohol, m.p. 195–196°. Due to the difficulty of working with the perchlorate salt, conversion to the methiodide salt was facilitated. The crystalline methiodide was recrystallized from methanol, m.p. 279–280°. The compound is optically inactive. The UV spectrum of the perchlorate as well as the methiodide salt show the presence of a phenanthrene nucleus. For the methiodide, the following absorption maxima are observed: $\lambda$ 213 m$\mu$ (log $\varepsilon$ 4.72), 230 m$\mu$ (log $\varepsilon$ 4.60), 251 m$\mu$ (log $\varepsilon$ 4.82), 257 m$\mu$ (log $\varepsilon$ 4.86), 278 m$\mu$ (log $\varepsilon$ 4.27), 302 m$\mu$ (log $\varepsilon$ 4.28), 312 m$\mu$ (log$\varepsilon$ 4.27), 344 m$\mu$ (log $\varepsilon$ 3.74) measured in methanol.

The infrared spectrum of the methiodide indicates the presence of an aromatic nucleus (3048 cm$^{-1}$) which is polycyclic in nature (doublet at 1600 and 1585 cm$^{-1}$). A strong peak at 1470 cm$^{-1}$ suggests the presence of $CH_2$ groups attached to the nitrogen which is substantiated by the multiplet at 6.75 $\tau$ for the four ethylene proton resonance in the NMR spectrum. A weak 1415 cm$^{-1}$ bond maybe due to the three methyl groups of the quaternary amino group. A sharp peak of nine protons at 6.95 $\tau$ signifies the presence of —N(CH$_3$)$_3$ group. The peak at 6.26 $\tau$ and 6.22 $\tau$ represent the two methoxyl groups at $C_3$ and $C_4$ as suggested by the 1382 cm$^{-1}$ band in the IR spectrum. Due to the low concentration of the solution which was used for the nuclear magnetic resonance measurement, the

aromatic protons were not clearly differentiated, however, the infrared peaks indicated one isolated hydrogen (860 cm$^{-1}$) located at $C_2$, two neighboring aromatic protons at $C_9$ and $C_{10}$ (820 cm$^{-1}$) and four adjacent hydrogens at $C_5$, $C_6$, $C_7$ and $C_8$ (755 cm$^{-1}$) of the phenanthrene nucleus.[1]

In 1965, Bick and Douglas [2] isolated atherosperminine from *Atherosperma moschatum* Labill (Monimiaceæ). They found out by comparison of the UV, IR, NMR spectra of the picrate and the methiodide of atherosperminine (III) that it is identical with the alkaloid 1-N,N-dimethylaminoethyl-3,4-dimethoxyphenanthrene, isolated by Cookes and Haynes [3] from *Cryptocarya*
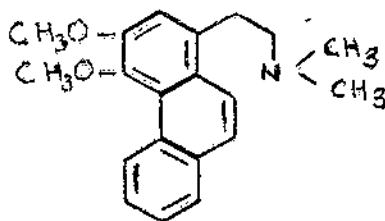


III

*angulata* C.T. White. The results of the spectral analyses ot the methiodide salt of *Anona muricata* L. indicate that it is atherosperminine methiodide.

The mass spectra of the methiodide gave a peak at m/e 309 which corresponds to $C_{20}H_{23}NO_2$ (the molecular formula of atherosperminine) and the base peaks at m/e 58 and m/e 41. According to Hesse, et al.[5] quarternary ammonium iodides do not register the molecular ion peak but eliminate methyl iodide (m/e 142) immediately or undergo thermal Hofmann degradation which may have been responsible for the base peak at m/e 58. The base peak at m/e 41 maybe represented as shown in the formula, which is also characteristic of the mass spectra of quaternary ammonium iodides. The peak at m/e 178 could only be due to the phenanthrene nucleus after the removal of all the substituents. The fragmentation pattern may be represented as follows:
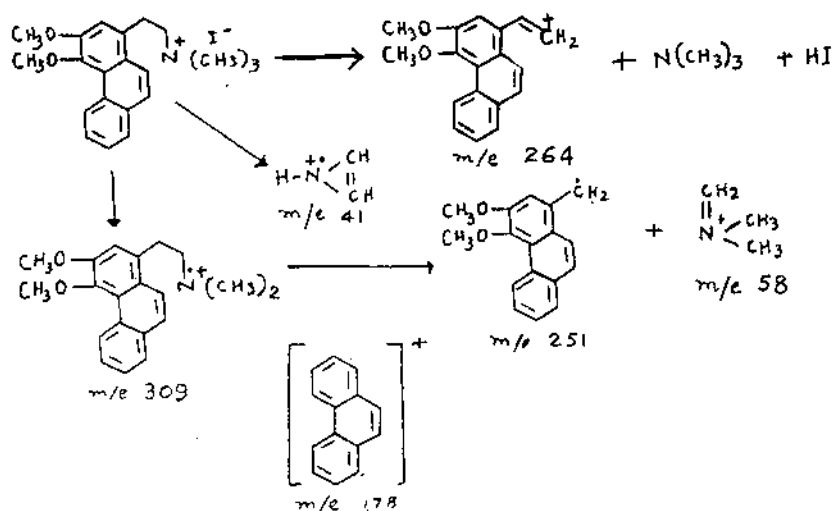
An additional proof to the effect that the nonphenolic alkaloid we have isolated is atherosperminine is that the picrate when mixed with the authentic sample melted at 186° showing no melting point depression. The authentic picrate was supplied by Dr. I.R.C. Bick of the Department of Chemistry of the University of Tasmania, Australia.

The occurrence of such phenanthrene alkaloids in so many plants is an indication of the possibility of Hofmann degradation of aporphine alkaloids in the cells which are usually found in the members of the Anonaceæ family.

### EXPERIMENTAL

*Extraction of total alkaloids.*—The alkaloidal extract was prepared from 21.7 kg of the powdered, dried bark of *Anona muricata* L. by first macerating the drug for 12 hours in 95-per cent ethyl alcohol, and then percolated exhaustively with the same solvent. The percolates were combined and concentrated under reduced pressure recovering most of the alcohol. Afterwards, the thick syrupy extract was evaporated on a water bath until free of alcohol giving a thick reddish brown residue weighing 1840 gm. The residue was digested with 1-per cent hydrochloric acid and filtered, removing most of the resinous matter. The acidic solution was shaken up with ammoniacal ether which extracted the total alkaloids. To further

effect the purification, the total residue was dissolved in 1-per cent hydrochloric acid and extracted again with ether after basifying, repeating the process twice. The yield of the crude total alkaloid is about 2.2 per cent.

*Isolation and identification of muricinine.*—The total tertiary extract was shaken with 2-per cent sodium hydroxide solution until no more phenolic base went to the alkaline solution. The alkaline solution was neutralized with concentrated hydrochloric acid, cooled and basified with ammonia and extracted with ether. The ethereal extract was concentrated, dried and taken up with chloroform and purified by passing through a column of neutral alumina. The perchlorate salt was prepared by dissolving the chloroformic residue in absolute alcohol and 70-per cent perchloric acid was added dropwise. The mixture was stored in a desiccator and after almost three weeks, crystals started to form. These were recrystallized from absolute alcohol, giving colorless prismatic crystals, m.p. 206–707° (dec.). The prismatic crystals gave green color with ferric chloride. With concentrated sulfuric acid in the cold, it gave greenish-blue color which turned blue to violet when heated to 130° and pink with Millon's reagent.

Specific rotation: $[\alpha]_D^{33} = +70°$ (c = 1, ethanol).

The UV spectrum gave: max (EtOH) 283 m$\mu$ (log $\varepsilon$ 3.72), $\lambda$ min (EtOH) 254 m$\mu$ (log $\varepsilon$ 2.78). The IR spectrum (KBr) showed absorption peak at 3300 cm$^{-1}$ (for hydroxyl group).

$C_{19}H_{23}O_4.N.HClO_4$ (431.06)

| | C | H | N | OCH$_3$ | N-CH$_3$ |
|---|---|---|---|---|---|
| Calculated | 53.29 | 5.59 | 3.26 | 14.45 | 3.40 |
| Found | 53.20 | 5.51 | 3.31 | 14.40 | 3.43 |
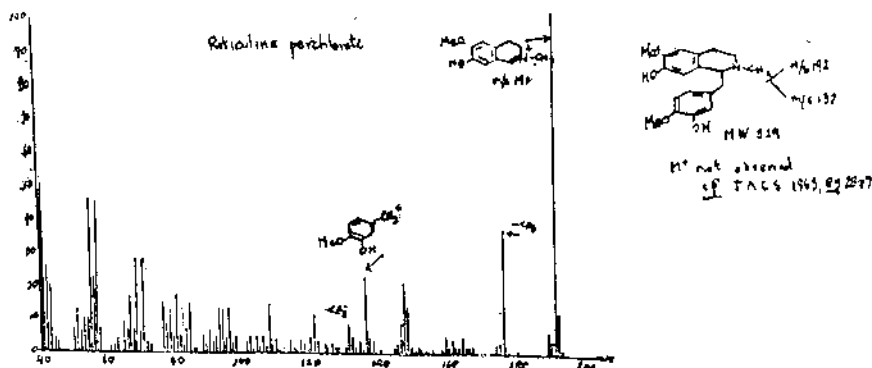


FIG. 1. *Mass spectrum of the perchlorate of the phenolic base.*

The mass spectra of muricinine reveals that most abundant peak or ion is at m/e 192 and m/e 177 and m/e 137 which are very characteristic of reticuline.

*Isolation and identification of atherosperminine.*—The residue containing the nonphenolic bases was dissolved in chloroform and passed through a column of neutral alumina activity 1 (E. Merck, Darmstadt) packed in an acid buret and eluted with chloroform. Fractions of 10–50 ml were collected. The fractions were combined and concentrated, the residue dissolved in absolute ethanol and a few drops of 70-per cent perchloric acid was added. Immediately brownish yellow crystals appeared. On recrystallization from 50-per cent ethanol, very light yellow crystal of perchlorate salt were obtained m.p. 195–196°.

Specific rotation: $[\alpha]_D^{30} = 0°$ (c = 1, acetone).

$C_{20}H_{23}O_2N.HClO_4.$

|  | C | H | N | OCH₃ |
|---|---|---|---|---|
| Calculated | 58.6 | 4.9 | 3.70 | 15.1 |
| Found | 58.2 | 4.9 | 3.81 | 16.0 |

*Preparation of atherosperminine methiodide.*—The perchlorate salt was suspended in enough water in a separatory funnel, alkalinified with ammonia water, and then extracted with ether. After distilling off the ether, the residue was dissolved in acetone and refluxed with an equivalent amount of methyl iodide. The crystalline methiodide was filtered and recrystallized from methanol, m.p. 279° to 280°. The yield was almost theoretical.

The UV spectrum of the methiodide (c=0.695 mg/10 ml methanol) λ max 213 mμ (log ε 4.72), 230 mμ (log ε 4.60), 251 mμ (log ε 4.82), 257 mμ (log ε 4.86), 278 mμ (log ε 4.27) 302 mμ (log ε 4.28), 312 mμ (log ε 4.27), 344 mμ (log ε 3.74) suggests the presence of phenanthrene nucleus. The IR spectrum in nujol gave 3048, 1600, 1585 cm⁻¹ (polycyclic aromatic nucleus), 1470 cm⁻¹ (—CH₂ groups attached to the nitrogen), 860 cm⁻¹ (one isolated aromatic proton) ; 820 cm⁻¹ (two neighboring aromatic hydrogens) ; 755 cm⁻¹ (four adjacent hydrogens). The NMR spectrum showed a peak for nine protons at 6.95 τ (—N(CH₃)₃) and the peaks at 6.22 τ and 6.26 τ represent the two methoxyl resonance and at 6.75 τ the four

ethylene proton resonance. The mass spectra register a peak at m/e 309 and the base peaks at m/e 58 and m e 41. Another characteristic peak is at m/e 178 (phenanthrene ring without any substitution).

## SUMMARY

The stem-bark of *Anona muricata* L. yielded three alkaloids. The phenolic base was isolated as a crystalline perchlorate salt, m.p. 206–207°, and was found to be identical with reticuline by UV, IR and mass spectral analysis.

The nonphenolic bases were separated by column chromatography on alumina. Nonphenolic base A was obtained as crystalline perchlorate salt, m.p. 195–196°. Its methiodide, m.p. 279–280°, proved to be identical with atherosperminine methiodide by analysis of its UV, IR, NMR, and mass spectra. Nonphenolic base B melts at 176–177°. The yield was so small to warrant any chemical or physical method of structural determination.

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# ILLUSTRATIONS

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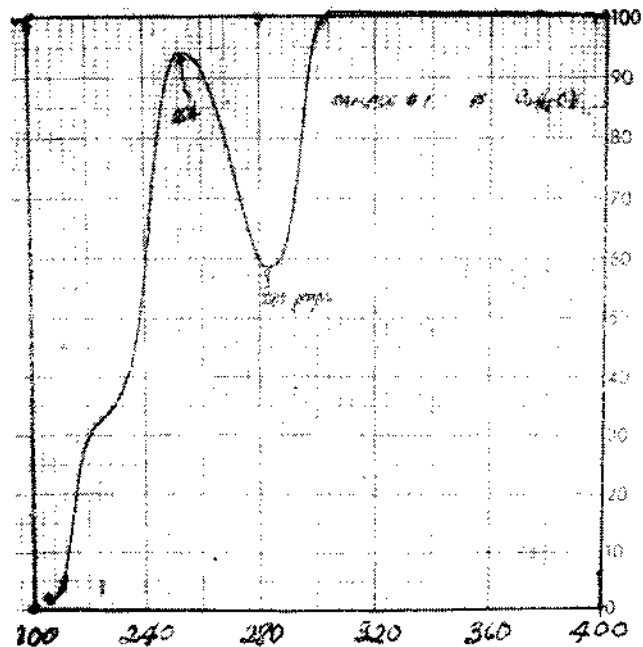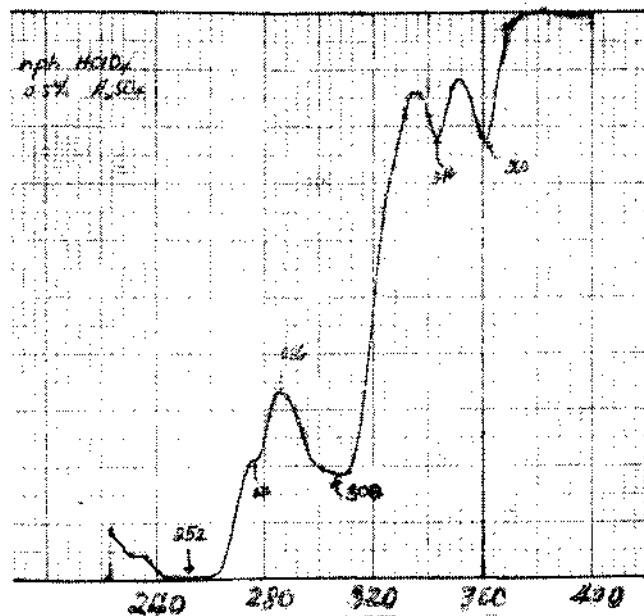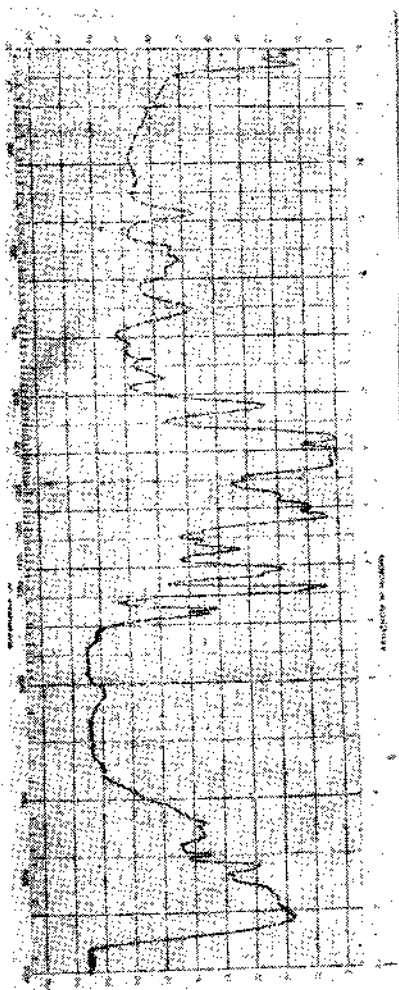
PLATE 1.

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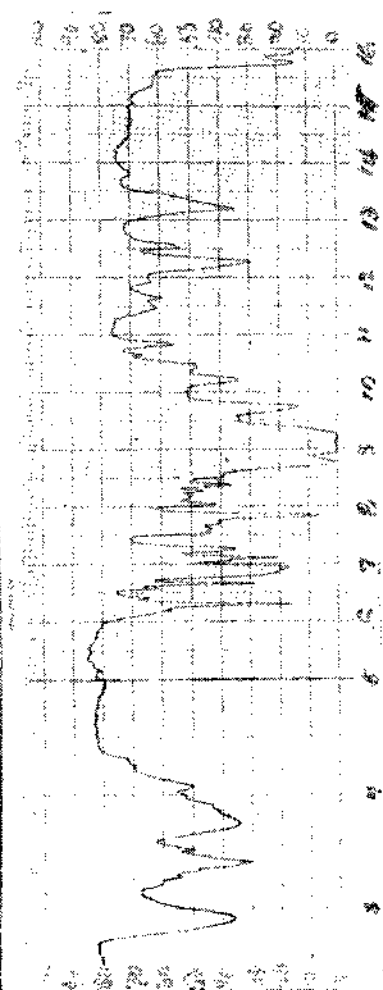
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PLATE 2.

# TWO ISOMERIC ALKALOIDS FROM THE STEM BARK OF KIBATALIA GITINGENSIS WOODS

By RIZALINA M. BERNAL-SANTOS

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THREE PLATES AND ONE TEXT FIGURE

Two isomeric alkaloids lanitine m.p. 184—186° C and lanitinine m.p. 223—225° C were isolated from the stem bark of *Kibatalia gitingensis* Woods,(5) known locally as "laniti" and belonging to the family Apocynaceæ. Three alkaloids have so far been isolated from this plant: kibataline or $3\alpha$ N,N–dimethylamino 20 S hydroxy–18 oic (–20) lactone 5 pregnene(2) (II) and N–methyl 20 epiparavallarine or $3\beta$ N,N–dimethylamino 20 R hydroxy-18 oic (–20) lactone 5 pregnene(3) (III) isolated from the leaves and bark, in 1965 by A. Cavé, et al. and gitingensine or $3\alpha$ amino 20 S hydroxy-18 oic (–20) lactone 5 pregnene(1) (IV) from the leaves by G. A. Santos. Elementary analysis and the molecular ion at m/e 373 in the mass spectrum established the molecular composition of the isomeric alkaloids as $C_{23}H_{35}O_2N$ (I).
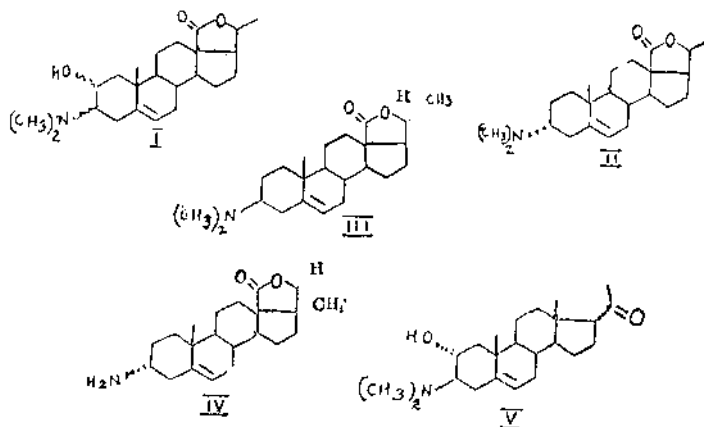


## EXPERIMENTAL

The finely ground stem bark was triturated with ammonia T.S. and extracted to exhaustion with ether in a Soxhlet ex-

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traction apparatus. Upon concentrating the ethereal extract (A) a sticky substance separated out which when extracted with acetone left a gummy residue. The acetone extract was concentrated and upon standing yielded white globules which gave negative alkaloidal test.

The concentrated ether extract (A) was extracted with 1-per cent hydrochloric acid and the aqueous extract (B) washed with ether and extracted with chloroform. The chloroformic extract (C) was washed with water, dried with anhydrous sodium sulfate and concentrated. From this, light amorphous white flakes separated out. This was filtered off by suction, washed with chloroform, dried and crystallized from acetone.

The alkaloidal hydrochloride was dissolved in distilled water, alkalinified with ammonia T.S. and extracted with ether. The ethereal extract was concentrated to crystallization and the product recrystallized from ether. The fraction which failed to dissolve in ether was recrystallized from acetone. Repeated fractional crystallization from ether and acetone was done until two crystalline products with definite melting points were obtained.

The mother liquor after removal of the hydrochlorides was evaporated to dryness. The residue dissolved in acetone gave a dark brown solution from which attempts to get crystalline products by column chromatography failed.

The aqueous acidic solution after extraction with chloroform was alkalinified with ammonia T.S. and extracted with chloroform. The chloroformic extract was concentrated and subjected to column chromatography. Likewise no crystalline products were obtained.

Paper chromatograms of the total hydrochlorides as well as the isolated free bases were run using Whatman paper No. 1 previously impregnated with 1 N HCl, methyl ethyl ketone as developing solvent and Dragendorff's reagent as detecting agent.

Thin-layer chromatograms of the free bases were also made using two systems: (1) methanol: acetone (4.3:7.6) as solvent system and 50-per cent sulfuric acid with heat and UV light for detection and (2) acetone: benzene: ammonium hydroxide

Schematic diagram of the isolation:

Finely ground stem bark
| triturate with ammonia T.S.
| extract exhaustively with ether

mare                    ethereal extract
                        | concentrate

sticky substance                    conc. ether extract (A)
| extract with acetone               | extract with 1 per cent HCl
acetone extract
| concentrate                aqueous extract B          ethereal sol.
                             | wash with ether
white globules        conc. acetone    | extract with chloroform
                          extract

                    aqueous solution         Chloroform    extract    (C)
                    alkalinify with          | wash with H$_2$O dry with
                    ammonia  T.S.            | anhydrous Na$_2$SO$_4$, con-
                    extract    with          | centrate
                    chloroform

aqueous solution          chloroform
                          ext. (D)

light amorphous flaked              conc. chloroform ext.
| filter off by suction             | evaporate to dryness
| wash with chloroform,            residue
| dry                               | dissolve in acetone
crude hydrochloride                 acetone extract
| crystallize from ace-
| tone
mixture of hydrochlorides
| dissolve in H$_2$O, alka-
| linify with ammonia
| T.S., extract with
| ether

ethereal extract                    aqueous solution
concentrate
crystals (free bases)

(32:32:1) as solvent system and Dragendorff's reagent as detecting reagent. For both systems silica gel (Stahl) was used as adsorbent.

Elementary analysis of the two isolated compounds were done. Their IR, NMR and mass spectra were run.

## RESULTS AND DISCUSSION

Percolation of the alkalinified drug with ethyl alcohol was also tried. This method proved to be more tedious since the alcoholic extract had to be defatted before extracting with acid. Besides, a smaller yield was obtained than the ether extraction which gave about 0.3 per cent yield of the crude hydrochlorides. Yield was found to vary also with different batches of plant materials.

The crude hydrochloride obtained was crystallized from acetone. Crystals obtained gave variable and widely ranging melting points so that a mixture was suspected. Attempts to separate the hydrochlorides by fractional crystallization failed. The bases were therefore liberated and crystallized fractionally until crystalline products with sharp melting points were obtained: lanitine, the ether-soluble base, as white platelets m.p. 184—186° C and lanitinine, the ether-insoluble base, as white crystalline needles m.p. 223—225° C. A third low-melting point product with very wide-melting range was obtained, the yield of which, however, was too small to warrant further work on it as of now.

Difficulty was encountered in the purification of the ether-insoluble base. Repeated washing with ether and recrystallization from acetone yielded products showing gradual increase in melting point which indicated presence of impurities.

Elementary analysis:

Lanitine:

| $C_{25}H_{37}O_3N$ | Calc. per cent | C 74.03 | H 9.46 | N 3.75 |
|---|---|---|---|---|
| | Found | 73.68 | 9.53 | 3.60 |

Lanitinine:

| $C_{25}H_{37}O_3N$ | Calc. per cent | C 74.03 | H 9.46 | N 3.75 |
|---|---|---|---|---|
| | Found | 74.51 | 9.87 | 3.74 |

Higher values obtained for carbon and hydrogen in lanitinine may be accounted for by the presence of a small amount of another compound as shown by thin-layer chromatography.

Paper chromatography of the hydrochloride mixture and the free bases failed to separate or differentiate the different compounds inasmuch as almost identical Rf-values were obtained: 0.59 for lanitine and 0.58 for lanitinine. Thin-layer chromatography using the first system gave elongated spots with no separation. The second system was more effective in giving a separation. The hydrochloride mixture gave three spots, the ether-soluble base gave a single spot while the ether-insoluble base gave more than one spot (Fig. 1).

Similar to kibataline the IR-spectra (Plate 1, figs. 1–2.) of the two alkaloids showed a strong carbonyl band at 1748 cm⁻¹ corresponding to the gamma-lactone function and shoulder bands at 2820, 2780 cm⁻¹ characteristics of steroidal alkaloids showing a dimethylamino group and absorption at 1667 and 795 cm⁻¹ showing unsaturation and similar to that found in $\triangle$5-pregnane derivatives. Similar to kurchiline [1] (V) lanitinine gives vibrations of an OH at 3420 cm⁻¹. From its IR spectrum lanitine shows a different OH from lanitinine. Bands at 1451 and 1376 cm⁻¹ typical of methyl groups at 1433 and 1406 cm⁻¹ indicative of methylenes are shown.
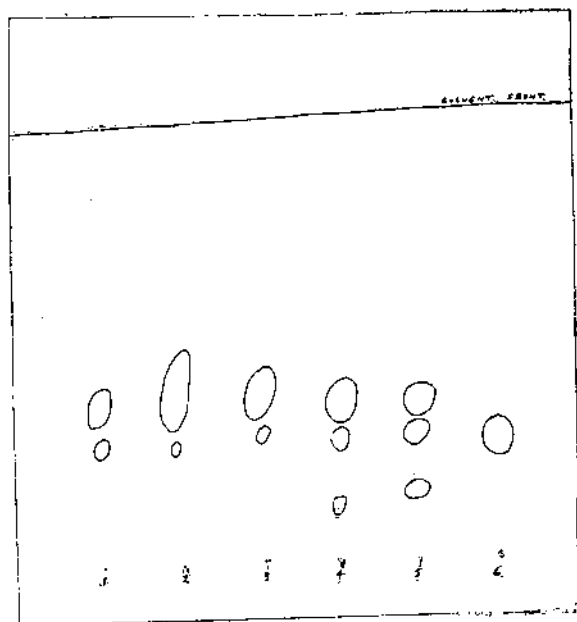


FIG. 1. TLC of (1) to (4) different fractions of ether-insoluble base, (5) total alkaloidal hydrochloride and (6) ether-soluble base, using silica gel G (Stahl) as adsorbent and acetone: benzene: ammonium hydroxide (32:32:1) as solvent system.

The mass spectra (Plate 2, figs. 3–4) of the two alkaloids are very similar with only slight intensity variations. Like kurchiline, two important peaks at m/e 58 (base peak) and m/e 100 are shown. The fragmentation can be illustrated as follows:
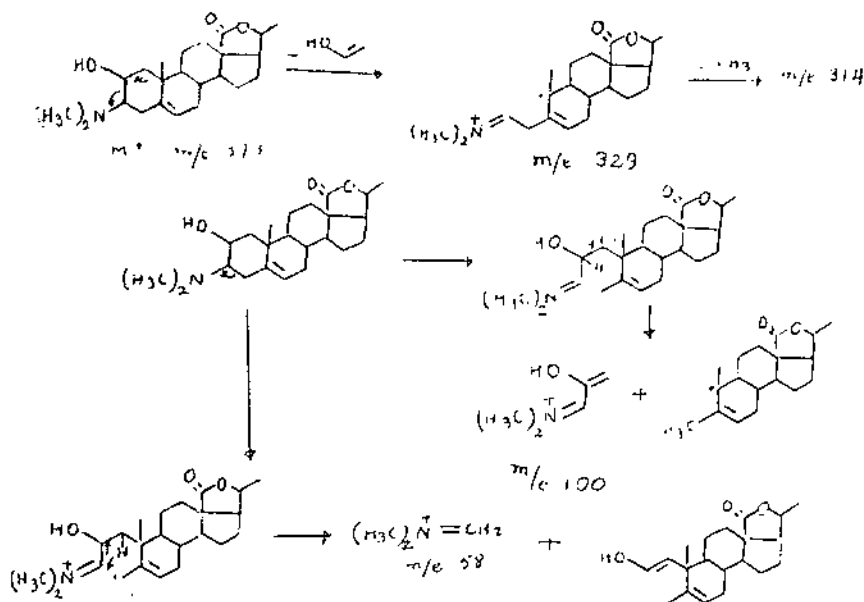


Ease in the rupture between carbons 2 and 3 is attributable to the presence of OH at 2. Double bond in 5,6 favors the rupture of bond at 3,4. This, taken in conjunction with molecular weight 373 and the characteristic gamma-lactone as shown by their IR spectra, indicates that lanitine and lanitinine are isomers with the formula $C_{23}H_{35}O_3N$ and the possible structure I.

Their IR spectra suggest that the difference is in the stereochemistry of the 2–OH and 3–N$(CH_3)_2$ groups. The sharp 3440 cm$^{-1}$ in lanitinine agrees well with the reported 3450 cm$^{-1}$ for a 2$\beta$–OH 3$\alpha$–N$(CH_3)_2$ orientation. The broad "bonded" 3400—3500 cm$^{-1}$ band in lanitine is close to the reported 3500 cm$^{-1}$ for a 2$\alpha$–OH 3$\beta$–N$(CH_3)$ structure rather than that for a 2$\beta$–OH 3$\beta$–N$(CH_3)_2$ structure.(4)   Comparing the NMR spectrum of lanitine (Plate 3, fig. 5): singlet of 3 protons at 1.1 (CH$_3$19), doublet of 3 protons at 1.38 (CH$_3$21), singlet of 6 protons at 2.33 [N$(CH_3)_2$ at 3], multiplet at 3.23

(protons $\propto$ to OH in 2), multiplet of one proton at 4.35 (H at $C_{20}$) and multiplet at one proton at 5.4 (ethylenic proton at 6): the NMR spectrum of lanitinine (Plate 3, fig. 6): singlet of 3 protons at 1.1 ($CH_3$ 19), doublet of 3 protons at 1.37 ($CH_3$ 21), singlet of 6 protons at 2.3 [$N(CH_3)_2$ at 3], multiplet at 3.33 (protons $\propto$ to OH in 2), multiplet of one proton at 4.35 (H at $C_{20}$) and multiplet of one proton at 5.4 (ethylenic proton at 6) with those of kurchiline and other kibataline derivatives further confirm the stereopositions of the hydroxyl and dimethylamine groups.(3, 4)

## SUMMARY

Two isometric alkaloids lanitine m.p. 184—186° C and lanitinine m.p. 223—225°C were isolated from the stem bark of *Kibatalia gitingensis* Woods. Chemical and physico-chemical methods of analysis have shown that lanitine is 2 $\alpha$ –hydroxy 3$\beta$– dimethylamino-20S hydroxy-18 oic (–20) lactone-5 pregnene while lanitinine is 2$\beta$-hydroxy 3$\alpha$ -dimethylamino-20S-hydroxy-18 oic (–20) lactone-5 pregnene.

## ACKNOWLEDGMENT

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# ILLUSTRATIONS

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PLATE 1.

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PLATE 2.

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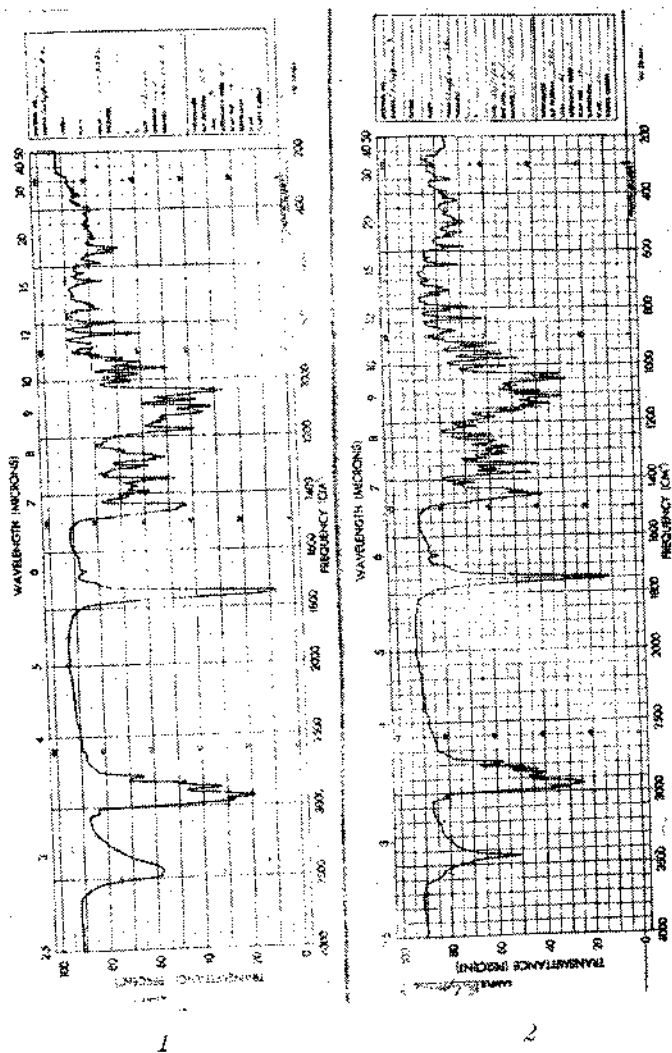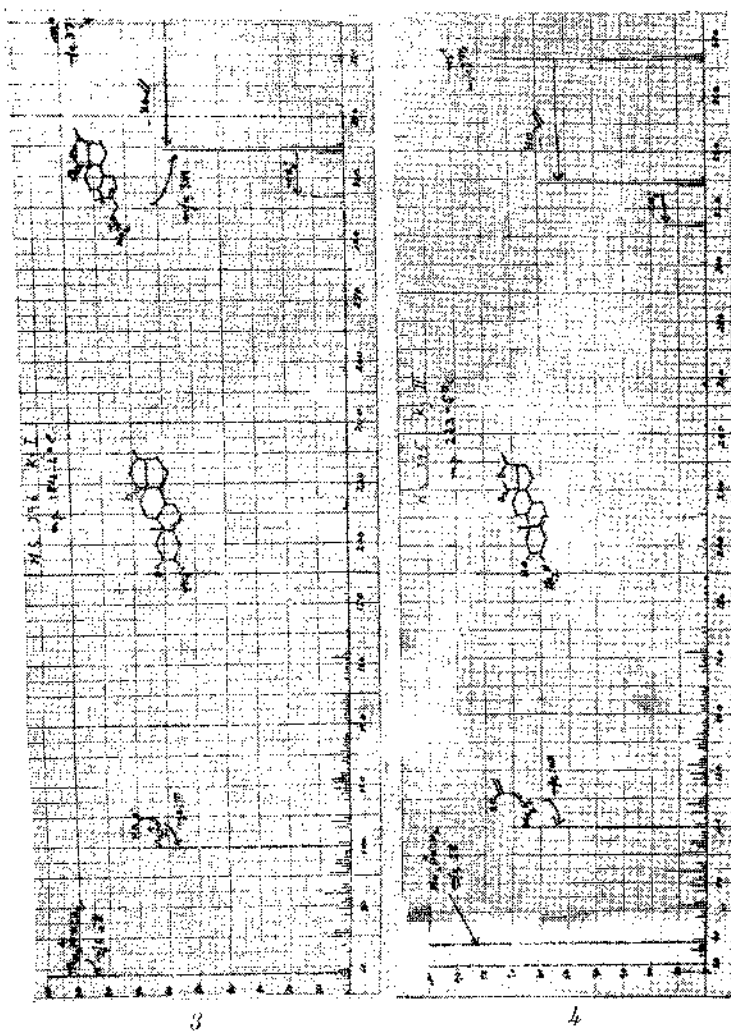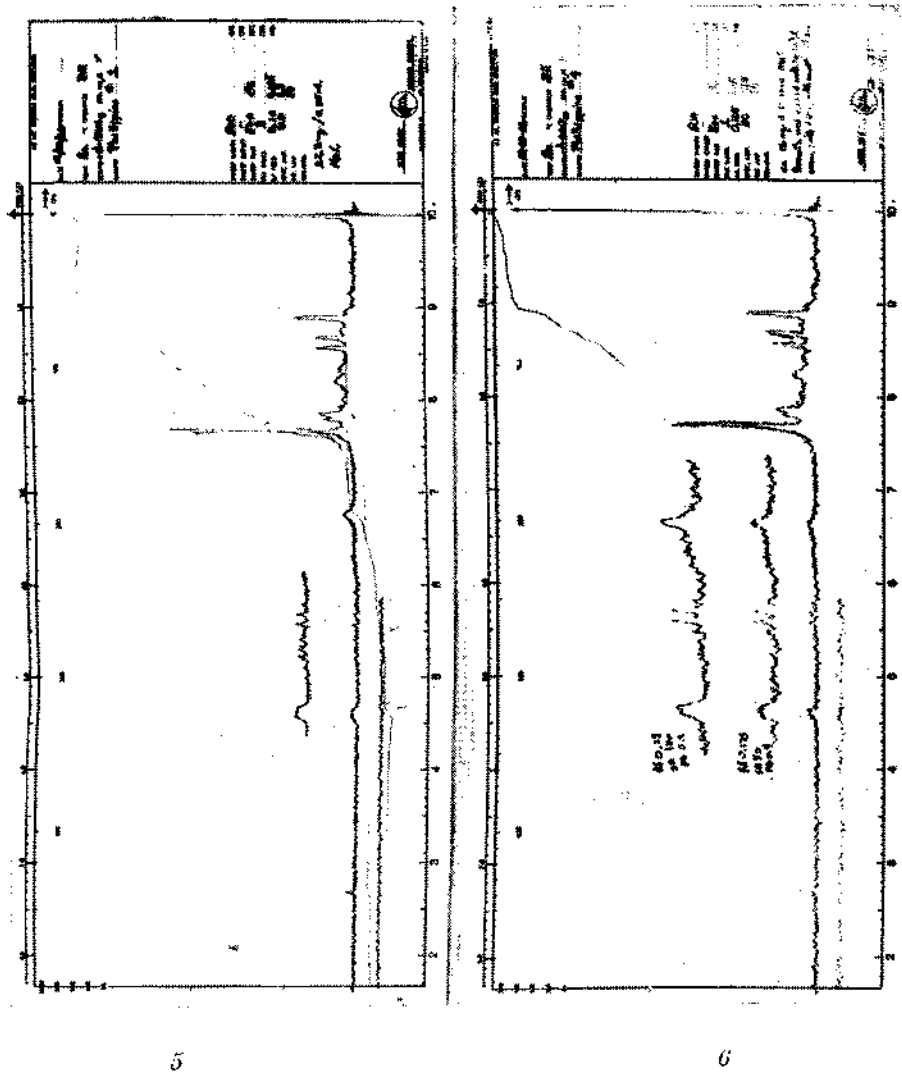
PLATE 3.

# AN EVALUATION OF THE MERTHIOLATE-IODINE-FORMALDEHYDE-CONCENTRATION TECHNIC AS A QUANTITATIVE METHOD OF STOOL EXAMINATION FOR SCHISTOSOMA JAPONICUM OVA *

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TWO TEXT FIGURES

## INTRODUCTION

It has been observed that in light infections, in advanced cases, or in patients previously treated for schistosomiasis but otherwise cured, *Schistosoma japonicum* eggs in the stool are so few that repeated stool examinations without the use of concentration technics often fail to demonstrate the eggs. Thus, attempts were made to determine the most efficient technic in the diagnosis of the disease as well as in making follow-up studies of post-treatment cases.

Blagg, et al. (1955) compared the merthiolate-iodine-formaldehyde-concentration (MIFC) technic with Ritchie's formalin-ether, the acid-ether of Telemann and others and found that MIFC resulted in the recovery of a greater number of eggs for each species of helminth than any of the other methods combined. Pesigan, et al. (1958) used the MIFC for screening large numbers of stool samples. In the same year, these workers made a comparative evaluation of the stool and the MIFC technics for the quantitative estimation of helminth eggs. It was noted that the stool technic was more appropriate for counting intestinal helminth ova but for schistosome eggs, the MIFC using 1 cm³ of feces was shown to be superior and easier to perform.

While the MIFC may be an efficient procedure for demonstrating and/or counting *S. japonicum* ova in stools, it is only relevant to know: (*1*) How consistent is the recovery of *S.*

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* Part of a thesis submitted to the Institute of Hygiene, University of the Philippines in partial fulfillment of the requirements for the degree of Master of Public Health.

*japonicum* ova from sample to sample or in the same sample after several centrifugations? and (2) How are *S. japonicum* ova distributed in the stool?

The manner by which 1 gram (or 1 ml by volume) of fecal sample is drawn for examination may also present an important source of variation in the egg count. *S. japonicum* ova may not be randomly distributed in the entire fecal mass so that count would then depend largely on the portion used for examination.

## MATERIALS AND METHODS

*Consistency of egg recovery.*—Two mushy-diarrheic [1] stool specimens were used to determine the consistency of recovery rate of *S. japonicum* ova using the MIFC technic after one or more centrifugations. One had a known high egg count and the other with relatively low count as determined by a previous examination. The stools were spread evenly over a plastic cover sheet and each one was divided into approximately 49 (7 × 7) equal parts. From the first specimen with a high egg count, 10 samples of 1 gram each were taken before stirring and another 10 after a thorough stirring to uniformly distribute the eggs in the entire sample. The table of random numbers was used in the selection of the samples. From the second stool specimen with a low count, ten samples were taken after thorough stirring. A total of thirty samples was used from the two specimens.

Each of the 30 samples was examined by the MIFC[2] technic. However, instead of the supernatant fluid including the layer of detritus being discarded after one centrifugation, this was transferred to another centrifuge tube and this process of shaking and centrifugation was repeated six times. This was done six times after it was noted in a previous trial that no more ova could be detected in the sixth sediment.

*Schistosome ova distribution in the stool.*—This study of the distribution of *S. japonicum* ova in the stool was conducted not only with the two mushy-diarrheic stools with high and relatively low egg counts but also with another pair of mushy-formed [3]

[1] Mushy-diarrheic stool is defined as one that will pour with difficulty; can be handled with a spatula [Scott (1938)].

[2] Centrifugation was carried out using the "IEC" Model CS centrifuge apparatus with horizontal head (19.8 cm radius at tip) giving 560 × G at 1,600 rpm.

[3] Mushy-formed stool is defined as one that will retain the cylindrical shape of the bowel but soft enough to be readily stirred [Scott (1938)].

stools, one with relatively high and the other with a low egg count. The mushy-diarrheic stools were spread evenly over a plastic cover sheet and each one was divided into approximately 36 (6 $\times$ 6) equal parts. Egg counts were made in 1-gram sample from each portion of the stool so that 36 samples from each of the two stool specimens were examined.

The mushy-formed stools were divided into ten cross-sectional segments. Each segment was divided into eight equal portions by four imaginary lines passing through the center. Each portion was further divided by a circular line around the center into an outer and inner section. Three random samples were obtained from the eight outer sections and another three from the eight inner sections of each segment. A total of 60 random samples was examined from each of the two mushy-formed stools.

On the basis of the results obtained from the first study, only the first three successive sediments from each of the mushy-diarrheic and mushy-formed stool samples were pooled and examined.

RESULT AND DISCUSSION

*Consistency of egg recovery.*—Tables 1, 2, and 3 show that the sixth sediment was negative for *S. japonicum* ova in 27 out of the total 30 samples studied. In the three samples in which the sixth sediment was still positive, only an insignificant number of eggs were recovered. It is, therefore, apparent that with six centrifugations, it could already be considered that the limit of recovery of eggs by the MIFC technic has been reached and that the total number of eggs counter per sample in all six sediments represent the total recoverable number (TRN) by the technic.

In the stirred stool with relatively high counts (Table 2), the ten random samples showed a mean TRN of 168.5 eggs with a range of 144 to 192. In the first sediment alone of the same samples, the mean number of eggs recovered was 69 with a range of 32 to 99. This mean number of eggs recovered in the first sediment represents 40.9 per cent of the TRN but varied as low as 18 per cent to as high at 63.1 per cent. The unstirred specimen likewise showed a wide ranged of egg recovery in the first sediment. In the stirred stool with low counts (Table 3), the 10 samples gave a mean TRN of 14.3

TABLE 1.—*Schistosoma japonicum ova recovery in 10 randomly selected samples from an unstirred mushy-diarrheic stool with relatively high egg counts.*

| Sample number | Total ova counted (1-6 added) | Ova recovery per sediment | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | 1st | | 2nd | | 3rd | | 4th | | 5th | | 6th | |
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| 1 | 58 | 42 | 72.4 | 13 | 22.4 | 3 | 5.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 79 | 49 | 62.0 | 18 | 22.8 | 11 | 13.9 | 1 | 1.3 | 0 | 0 | 0 | 0 |
| 3 | 124 | 97 | 78.2 | 17 | 13.7 | 10 | 8.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 140 | 124 | 88.6 | 13 | 9.3 | 3 | 2.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 89 | 75 | 84.3 | 13 | 14.6 | 1 | 1.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 118 | 90 | 76.3 | 15 | 12.7 | 11 | 9.3 | 1 | 0.8 | 1 | 0.8 | 0 | 0 |
| 7 | 110 | 76 | 69.1 | 22 | 20.0 | 10 | 9.1 | 2 | 1.8 | 0 | 0 | 0 | 0 |
| 8 | 95 | 47 | 49.5 | 44 | 46.3 | 3 | 3.2 | 1 | 1.1 | 0 | 0 | 0 | 0 |
| 9 | 89 | 56 | 62.9 | 22 | 24.7 | 7 | 7.9 | 4 | 4.5 | 0 | 0 | 0 | 0 |
| 10 | 80 | 43 | 53.8 | 30 | 37.5 | 5 | 6.3 | 1 | 1.2 | 1 | 1.2 | 0 | 0 |
| Total | 982 | 699 | 71.2 | 207 | 21.1 | 64 | 6.5 | 10 | 1.0 | 2 | 0.2 | 0 | 0 |

TABLE 2.—Schistosoma japonicum ova recovery in 10 randomly selected samples from a stirred mushy-diarrheic stool with relatively high egg counts.

| Sample number | Total ova counted (1–6 added) | Ova recovery per sediment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | | 2nd | | 3rd | | 4th | | 5th | | 6th | |
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| 1 | 190 | 71 | 37.4 | 102 | 53.7 | 17 | 8.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 157 | 79 | 50.3 | 71 | 45.2 | 7 | 4.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 178 | 32 | 18.0 | 126 | 70.8 | 6 | 3.4 | 7 | 3.9 | 3 | 1.7 | 4 | 2.2 |
| 4 | 161 | 68 | 42.2 | 79 | 49.1 | 4 | 2.5 | 6 | 3.7 | 4 | 2.5 | 0 | 0 |
| 5 | 160 | 50 | 31.3 | 98 | 61.3 | 4 | 2.5 | 8 | 5.0 | 0 | 0 | 0 | 0 |
| 6 | 157 | 99 | 63.1 | 47 | 29.9 | 4 | 2.5 | 6 | 3.8 | 1 | 0.6 | 0 | 0 |
| 7 | 144 | 61 | 42.4 | 73 | 50.7 | 6 | 4.2 | 3 | 2.1 | 0 | 0 | 1 | 0.7 |
| 8 | 158 | 61 | 38.6 | 50 | 31.6 | 14 | 8.9 | 23 | 14.6 | 7 | 4.4 | 3 | 1.9 |
| 9 | 192 | 88 | 45.8 | 94 | 49.0 | 8 | 4.2 | 2 | 1.0 | 0 | 0 | 0 | 0 |
| 10 | 188 | 81 | 43.1 | 88 | 46.8 | 7 | 3.7 | 9 | 4.8 | 3 | 1.6 | 0 | 0 |
| Total | 1,685 | 690 | 40.9 | 828 | 49.1 | 77 | 4.6 | 64 | 3.8 | 18 | 1.1 | 8 | 0.5 |

TABLE 3.—*Schistosoma japonicum ova recovery in 10 randomly selected samples from a stirred mushy-diarrheic stool with relatively low counts.*

| Sample number | Total ova counted (1-6 added) | Ova recovery per sediment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | | 2 nd | | 3rd | | 4th | | 5th | | 6th | |
| | | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent | Number | Per cent |
| 1 | 12 | 9 | 75.0 | 3 | 25.0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | 17 | 15 | 88.2 | 1 | 5.9 | 1 | 5.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | 16 | 15 | 93.8 | 1 | 6.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 14 | 8 | 57.1 | 5 | 35.7 | 1 | 7.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 13 | 11 | 84.6 | 1 | 7.7 | 1 | 7.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | 14 | 8 | 57.1 | 6 | 42.9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 15 | 13 | 86.7 | 2 | 13.3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | 13 | 12 | 92.3 | 0 | 0 | 1 | 7.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | 15 | 12 | 80.0 | 3 | 20.0 | 1 | 7.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 14 | 11 | 78.6 | 2 | 14.3 | 0 | 0 | 0 | 0 | 1 | 7.1 | 0 | 0 |
| Total | 143 | 114 | 79.7 | 24 | 16.8 | 4 | 2.8 | 0 | 0 | 1 | 0.7 | 0 | 0 |

with a range of 12 to 17. In the first sediment alone of the same stool, the mean number of eggs was 11.4 with a range of 8 to 15. The mean number of eggs recovered in the first sediment represents 79.7 per cent of the TRN and varied from 57.1 to 93.9 per cent.

Using the Chi-square test, it was shown that the recovery rate of eggs in the stirred and unstirred specimens with high counts differed significantly in the samples examined. However, in the stirred samples with relatively low counts, the difference in recovery rates in the first sediment of the ten samples examined was shown not to be significant. It can be deduced that because of the significant variations in egg recovery from the stool with high counts, the examination of the first sediment alone will not give satisfactory results. For the stool with low counts, the examination of the first sediment may be sufficient. The reference here to a high and low counts are relative since there is still no accepted range in determining what is a high or a low count, aside from the fact that only two stools with different levels of egg counts were used. It would seem preferable not to confine the examination to only the first sediment even when examining stools of low counts.

Recovery rates in the second to the third sediments of the stool with relatively high counts also showed wide variations in both unstirred and stirred fecal specimens. In the second sediment of the unstirred fecal specimen, 21.1 per cent of the eggs were recovered with a range of 9.3 to 46.3 per cent in the different samples examined. Recovery rates in the third sediment were still relatively high ranging from 1.1 to 13.9 per cent but the fourth to the sixth sediments yielded insignificant total counts of 1, 0.2, and 0 per cent, respectively.

The stirred specimen from the same stool gave a higher recovery rate in the second sediment compared to the first with a per cent recovery rate of 49.2 (range of 29.9 to 70.8 per cent) and 40.9 (range of 18 to 63.1 per cent), respectively. The third yielded a total of 4.6 per cent (range of 2.5 to 8.9 per cent). The fourth to the sixth sediments likewise showed corresponding low recovery rates of 3.8, 1.1, and 0.5 per cent.

The combined first and second sediments and the combined first three sediments of the stirred specimen with high counts gave no consistent recovery rates. For all practical purposes, egg counts in these sediments may already suffice for ova

quantitation since a total of 95 per cent or more of the eggs can already be recovered in them.

*Schistosome ova distribution in the stool.*—The egg counts from the mushy-diarrheic stool with a relatively high and low count revealed a range of 357 to 1,109 and 26 to 81 eggs, respectively (Tables 4 and 5). The wide range in egg counts may indicate lack of homogeneity in ova distribution in the mushy-diarrheic stools examined.

TABLE 4.—*Distribution of Schistosoma japonicum egg counts in 36 samples from a mushy-diarrheic stool with relatively low counts.*

| Egg counts | Frequency |
|---|---|
| 20–29 | 3 |
| 30–39 | 9 |
| 40–49 | 11 |
| 50–59 | 6 |
| 60–69 | 2 |
| 70–79 | 4 |
| 80–89 | 1 |
| Total | 36 |

TABLE 5.—*Distribution of Schistosoma japonicum egg counts in 36 samples from a mushy-diarrheic stool with relatively low counts.*

| Egg counts | Frequency |
|---|---|
| 300–399 | 2 |
| 400–499 | 4 |
| 500–599 | 6 |
| 600–699 | 10 |
| 700–799 | 6 |
| 800–899 | 5 |
| 900–999 | 1 |
| 1,000–1,099 | 1 |
| 1,100–1,199 | 1 |
| Total | 36 |

The mushy-formed stool with high egg counts showed an uneven distribution of eggs. In this particular stool, a more or less diminishing count was obtained from the first to the last stool segment starting from the first portion that came out. Figure 1 shows that the first segment has the highest average count of 929 eggs per gram which decreased to 170 eggs and more or less maintained up to the last portion of the stool.
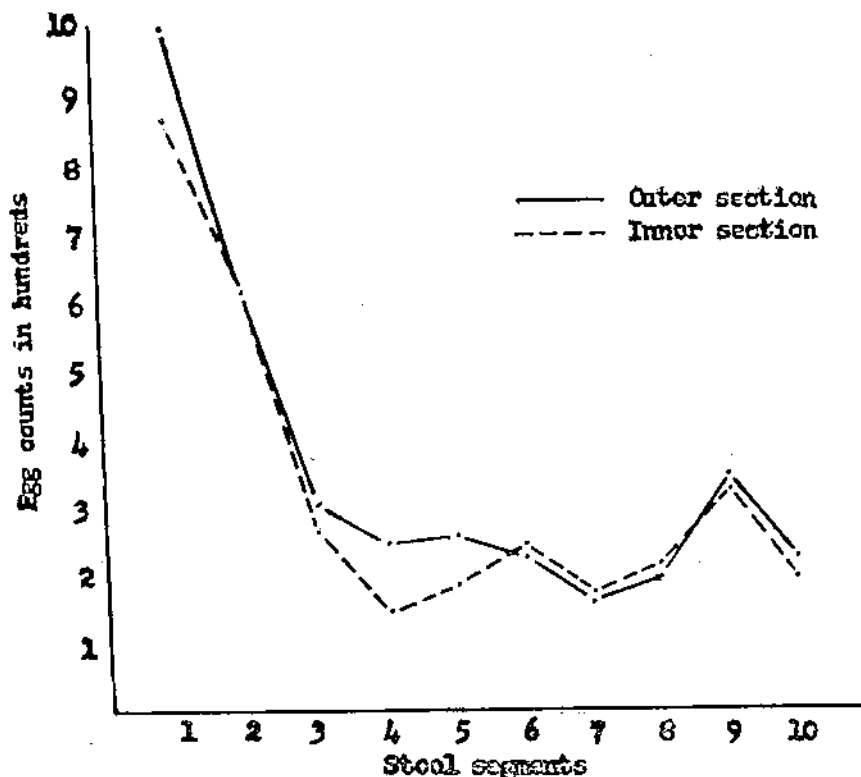
FIG. 1. *Schistosoma japonicum* egg counts per gram in the 10 successive segments of a mushy formed stool with hight counts.

The outer and inner portions of each segment showed also a more or less diminishing pattern with counts ranging from 996 to 163 and 861 to 146 eggs, respectively. The mean difference in egg counts in the outer and innersections was found to be 33 which turned out to be statistically significant.

Egg counts from the mushy-formed stool with a lower count likewise showed a higher number of eggs in the first segment in both the outer and inner sections with a mean egg count per gram of 97 and 39, respectively. The latter segment contained as low as 16 eggs in the outer and 11 eggs in the inner sections (Figure 2). Statistical analysis showed that the mean difference of 10.9 in the outer compared to the inner sections was not significant. However, the pattern of the difference from segment to segment points to a suggestive signi-

ficance in the difference between the outer and inner sections.
This might have been more evident had the size of the sample
been increased.

The results show that in mushy-diarrheic and mushy-formed
stools examined, *S. japonicum* eggs are not randomly distributed.
This differed from the distribution of ascaris, trichuris and
hookworm eggs which were reported to be randomly dispersed
[Martin (1965)]. This could be expected especially in ascaris
and hookworm infections where the main habitat of the worms
is the small intestine. In the case of *S. japonicum* infection,
proctoscopic and postmortem examinations among the American
military personnel and members of the Australian Air Force
Unit who had acquired the infection in Leyte late in 1944
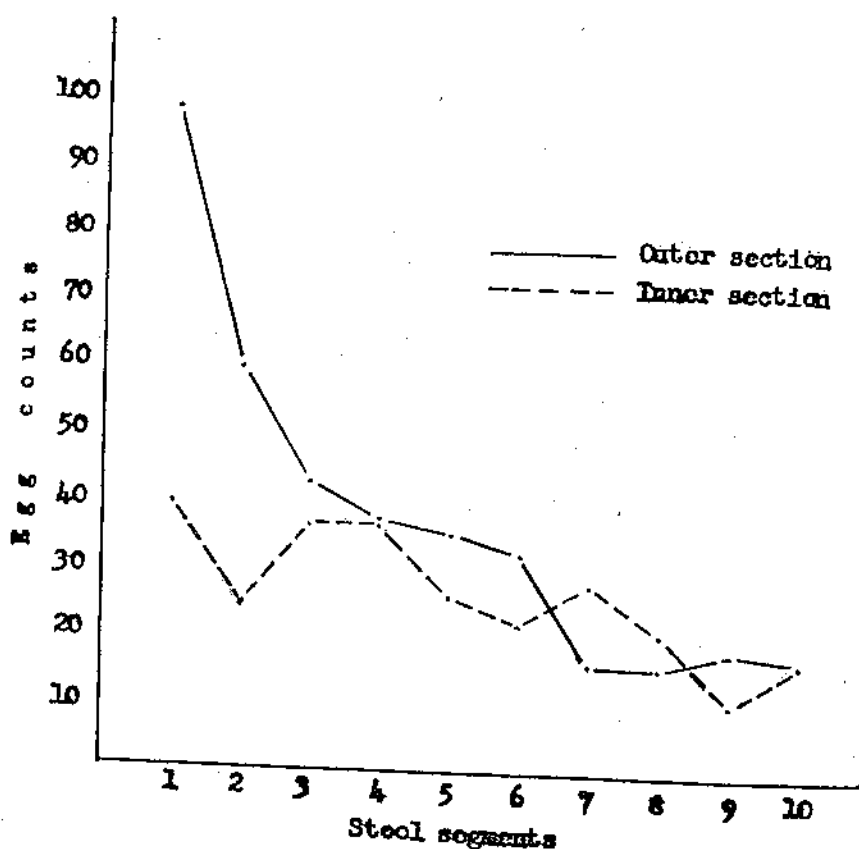
FIG. 2. *Schistosoma japonicum egg* counts per gram in the 10 successive
segments of a mushy-formed stool with low counts.

and early 1945 showed that some of the worms were in the inferior messenteric radicles and at times at the rectal venules discharging eggs and/or causing the formation of pseudotubercles [Most, et al. (1950) and Hunter, et al. (1951)]. This was aptly reviewed by Faust and Russel (1957) who stated that while early intestinal lesions in schistosomiasis japonica was supposedly confined primarily to the small bowel, the above reported evidences indicate that this is no longer true. The lack of homogeneity in the stools examined may be attributed to lesions in the lower intestinal tract.

Unless this nonhomogenous distribution of *S. japonicum* eggs in the stool, including the lack of consistency in recovery rates from the first sediment are overcome, the use of the MIFC as a quantitative method for *S. japonicum* egg count may not give reliable results.

### SUMMARY AND CONCLUSIONS

A study was carried out to evaluate the reliability of the MIFC technic as a quantitative method of stool examination for *S. japonicum* ova. Consistency of recovery rates from the sediments of 1-gram stool samples was determined. *S. japonicum* egg distribution in the different portions of the stool was likewise made.

The results showed that there was no consistent recovery rates in the sediments of the different samples examined from the stool with high egg counts. However, about 95 per cent or more of the eggs could be recovered in the combined three sediments from the same fecal sample indicating that the examination of the first three sediments would, for practical purposes, probably suffice for *S. japonicum* ova quantitation. On the other hand, variation in the recovery rates from the first sediment of the stool with a relatively low count turned out not significant.

A wide range in the egg counts was noted in the mushy-diarrheic stool which may indicate lack of homogeneity in egg distribution. In the mushy-formed stools examined, the first portion was found to contain the greatest number of eggs which diminished and more or less maintained from the middle part to the last portion. Egg counts in the outer and inner sections of the stool with high counts varied significantly. In the one with a relatively lower count, the mean difference was not

significant. However, the pattern of difference from segment to segment points to a suggestive significance in egg counts between the outer and inner sections. This might have been more evident had the size of the sample been increased.

In view of the findings presented above, it is felt that further evaluation should be made on the reliability of examining at least the three successive sediments from each gram of a well stirred 24-hour stool using the modified MIFC technic described above as a possible approach in *S. japonicum* egg counting.

## ACKNOWLEDGMENT

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# BOOK REVIEW

Books reviewed in this section represent a selection from those received occasionally by the National Institute of Science and Technology and the Philippine Atomic Energy Commission, two sister agencies under the National Science Development Board.

A summary of the morphology and ecology of 27 species of figs recorded in Hong Kong and a systematic enumeration of the fig wasps associated with 14 species that flowered during the course of study.

Dr. Hill, who is an entomoligist, apparently has the ultimate aim of studying in detail the systematics of the insects that live in close association with figs. As stated in the introduction of his work, this paper is the first of a series on the figs and fig wasps of Hong Kong which is perhaps a more appropriate title for the book although only 20 per cent of the entire work is devoted to fig wasps. The work on the host plants, however, is a prerequisite to the study of the insects.

A taxonomic key to mature and fruit-bearing figs is presented. The book is lavished with fine illustrations consisting of 65 photographs and 178 text figures. With the aid of these illustrations and referral to the good morphological descriptions, identification of the different species of figs is made very easy. Each fig species is described fully and observations on the habitat, seasonal occurrence of fig crops, leaf fall, and geographical distribution are noted. Previous records of insects in association with each fig species are listed. The pollination of the fig flowers by symbiotic wasps is clearly explained.

The maps and distribution records do not show that *Ficus elastica* and *Ficus pumila* are found in the Philippines. As a matter of fact these two are very common in homes and gardens. For easy reference, the synonyms of the figs should have been listed before the species description; instead, a table listing previous names used for Hong Kong is appended in the end, following the 18-page bibliography.

As an introductory part of the study of the fig wasps of Hong Kong, the author included a catalogue of the world genera of fig wasps. Of significance are the erection of one new subfamily and one new tribe in the family Torymidæ and the sinking into synonymy of 14 chalcid genera. However, the reasons for making the synonymies should have been stated.

With these foundations laid, the subject matter of Hong Kong fig wasps was introduced and presented but very concisely in tabulated form. The total number of chalcid wasps (pollinators and their parasites) that issued from mature fruits of 14 fig species is 65. These wasps belong to the families Agaonidæ, Torymidæ, Pteromalidæ, Eurytomidæ, Ormyridæ, and Eulophidæ. The generic determinations have been made but not all of the specific determinations. It was found that the vast majority of wasps examined were completely host specific. It is anticipated that the systematic and ecological treatments of the 65 chalcid species will be treated in subsequent publications.

Despite its limited geographic coverage, biologists will find this piece of research interesting and somehow useful. It demonstrates the high degree of adaptive radiation in the genus *Ficus*, the evolution and dispersal of both plants and insects, and the correlation between the plant and insect systematics. More studies of this kind should be conducted especially in the tropics with Dr. Hill's work serving as a model—C.R.B.

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[New names and new combinations are printed in italics.]

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